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CORRIGENDA

Volume 153, page 508. Line 5: The designation C_0^+ should read C_0^*.
 Line 10: The equation should read $C_t^* = C_0^* e^{-Rt}$.
Volume 154, page 428. In title and throughout text, "Herring-Breur" should read "Hering-Breuer".

ELECTROPHRENIC RESPIRATION[1]

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PRESENT methods of artificial respiration, with the exception of the body-enclosing respirators (1, 2), introduce unphysiologic factors which, under various circumstances and for various reasons, are undesirable. Beecher, Bennett and Bassett (3) have recently examined the adverse effects of elevated intratracheal pressure on the circulation in 'shock' states. A detailed critique of the various methods of artificial respiration will not be presented here. Suffice it to say that, if the diaphragm could be made to contract and relax in a controllable manner, a method of artificial respiration more nearly simulating natural breathing would be available. Such a method would obviate the necessity for the expense and bulk of the body respirator.

It has long been known that the response of a muscle to the stimulation of its nerve is in direct proportion to the intensity of the applied stimulus (4, 5). Theoretically, therefore, a continuously varying, undulating stimulus applied to a nerve should result in a continuously varying, undulating contraction of the innervated muscle. Since an electrical stimulus applied to a nerve can be readily varied in contour and rate, the production of artificial respiration by appropriate stimulation of the phrenic nerve seemed a logical possibility. This report describes the application of such a technique to the production of artificial respiration in several species of laboratory animals.

METHOD

The experiments were performed on the cat, dog, monkey and rabbit. The anesthesia used is indicated in table 1. Barbiturates were, for the most part, not used because of the difficulty of avoiding some degree of respiratory depression. In one experiment (*no. 17*) nembutal was used for

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anesthesia and respiratory depression was minimized by the use of small doses. Nembutal was used in large doses in the one experiment (*no. 18*) in which it was desired to produce respiratory depression due to barbiturate poisoning.

Blood samples were collected under oil or over mercury and analyzed immediately for O_2 and CO_2 tensions according to the method of Riley (6). Determinations were done in duplicate. Minute volumes were obtained by collecting expired air in a spirometer.

An electrode (or, in some cases, two electrodes) was brought into contact with the main trunk of the phrenic nerve low in the neck posterior to the subclavian vein, at which point the phrenic filaments from the various levels of the cervical outflow join together or lie in close proximity (fig. 1). After incision of the skin, a cleavage plane was developed between the external jugular vein and the sternocleidomastoid muscle; the latter was retracted medially exposing the uppermost filament of the phrenic nerve in its position on the posterior cervical fascia. This filament lies lateral to and parallel to the carotid sheath; it was followed downward and the point found at which the phrenic filaments converged. Each filament was tested electrically to be sure of its function. Proper electrode implantation was most easily accomplished in the dog, next most easily in the cat. It was difficult to accomplish proper electrode application in the monkey because of the very short neck, high clavicle and diffuse outflow that makes up the phrenic bundle. The latter forms a single trunk quite low in the neck, an arrangement very different from that found in man. Rabbits were used in the preliminary experiments and although it is feasible to produce electrophrenic respiration in that species, the delicacy of the nerve, the low blood volume and the difficulty of doing repeated arterial punctures make it unsuitable for experiments involving blood gas analysis. The difficulty of abolishing respiration is likewise greater in the rabbit.

Several different ways of making and implanting the electrode were tried. The following is the best method developed thus far. Four no. 34, pure silver strands were wound spirally until they formed a fine, multiple-strand wire; this was cut into 12-inch lengths, each to serve as both electrode and lead wire. Each unit was covered snugly with polyethylene tubing (free of plasticizer) with an inside diameter of 0.34 inch and an outside diameter of 0.48 inch. Three quarters of an inch of the wire at each end was left bare, one end to serve as the stimulating electrode, the other to be connected to the source of current. A flat piece of pure polyethylene sheeting (free of plasticizer), 2 inches by ⅜ inch by 0.0015 inch, was placed under the phrenic trunk (fig. 1). One bared end of the silver wire was placed around the nerve and twisted on itself so that the phrenic nerve could not escape contact with the electrode in some portion of its perimeter. A silk suture was used to secure the distal to the proximal limb of the loop. An additional silk suture then secured the insulating sheeting to the electrode and to itself. In this way the phrenic nerve could not escape contact with the electrode but was not compressed by it and the latter could not deliver an impulse to any other structure. The other end of the silver wire was threaded onto a surgical needle and led out through the skin at some distance from the original wound. The wound was then closed. In order to complete the circuit, an indifferent electrode in the form of a hypodermic needle or ECG plate was applied at some convenient distant point on the body.

This arrangement was found to be most satisfactory for several reasons:

1) The loop lies loosely about the nerve and does not compress it although it is always in contact with it.

2) Having a single electrode in contact with the nerve avoids the occurrence of the possible ischemia in that segment of nerve which would otherwise lie between two electrodes.

3) The fine lead wire can be threaded onto a surgical needle and made to emerge from the neck at a considerable distance from the incision so that the latter can be closed. Using only one electrode instead of two diminishes whatever undesirable effects may attend the purposeful implantation of a foreign body.

4) If both phrenic nerves are stimulated, the electrode on one nerve serves as the indifferent electrode for the opposite side and vice versa so that the externally applied electrode can be dispensed with; indeed, two electrodes on each nerve would be superfluous under those conditions.

5) Polyethylene is a good insulator for both the silver wire and nerve because of its demonstrated inertness in contact with nervous tissue (7).

The current used for nerve stimulation was delivered by a Grass Stimulator, although a much

simpler and less expensive device could be used, now that the limits of frequency, duration and voltage are established. Individual stimuli having a duration of 2 milliseconds and delivered at a frequency of 40 per second were found suitable for phrenic nerve stimulation. The current was fed through a rotating potentiometer which delivered a voltage that varied regularly between 0 and about 3 volts. Such a method of stimulation makes the diaphragm perform a reasonable imitation of its normal respiratory motion. The rate at which the lever arrangement actuates the rotating potentiometer can be varied from 0 to 60 per minute either by means of a friction clutch or a variable speed motor control so that the respiratory rate can be similarly varied. The rate and shape of the rotating potentiometer motion can be varied by adjustments of lever length and height. The mechanism is simple and reliable.

The abolition of spontaneous respiratory activity was accomplished in one of three ways:

1) The atlanto-occipital membrane was opened and a no. 18-needle inserted through the tela choroidea into the fourth ventricle. A segment of fine polyethylene tubing was fed through the needle while the latter was withdrawn leaving the tubing in place. Injections of 1 to 2 cc. of 1 per cent procaine hydrochloride caused complete cessation of spontaneous respiratory effort in from one to two minutes, an effect which lasted for variable periods of time after which additional injections could be conveniently made through the previously placed tubing. The method will be described in detail in a separate communication (8).

2) Electrophrenic respiration, set at a rate and minute volume similar to but slightly in excess of the animal's own rate and minute volume, caused the animal to cease spontaneous respiratory activity. This sometimes occurred after only the first or second electrically induced diaphragmatic contraction. The explanation of this phenomenon is not entirely clear, but it could be produced in all but a few instances. Simple observation of the animal as well as pneumograms taken during the transition period made clear the absence of spontaneous respiratory effort when the animal was put on electrophrenic respiration (15). The mechanism of this central respiratory inhibition will be reported separately (16).

3) Large doses of nembutal intravenously were used in one experiment (no. 18) for respiratory depression in order to simulate barbiturate poisoning. Nembutal was also used in one prolonged experiment but is not well suited to that purpose.

Pneumotachograms (air flow velocity patterns) were taken with the instrument devised by Silverman (9) and Silverman and Whittenberger (10).

The experiments were designed to yield data on four points. 1) To ascertain whether normal minute volumes and blood gas tensions could be achieved with sub-maximal stimulation of one phrenic nerve. 2) To test the reserve of the method by measuring minute volumes and blood gas tensions during maximal stimulation of one and both phrenic nerves. 3) To clarify the relationship between peak voltage applied to the phrenic nerve and the corresponding minute volumes. 4) To ascertain whether the method could maintain respiration during prolonged acute experiments.

<div align="center">RESULTS</div>

1. *Respiratory Minute Volumes and Blood Gas Partial Pressures Under Spontaneous and Electrophrenic Respiration.* The values of respiratory minute volume and arterial oxygen and carbon dioxide tensions during spontaneous breathing and during stimulation of one or both phrenic nerves are compared in table 1. It is apparent from these data that adequate ventilation can be readily accomplished in the absence of spontaneous respiration by submaximal stimulation of one phrenic nerve.

2. *Maximal Ventilation with One or Both Phrenic Nerves. Experiment 13* in table 1 compares the spontaneous minute volumes and arterial tensions of oxygen

and carbon dioxide with those values obtained during maximal stimulation of one phrenic nerve, after spontaneous respiration had been abolished. *Experiments 16* and *18* demonstrate the several-fold increase in ventilation that can be accomplished with bilateral stimulation and the resulting rise in oxygen pressure and fall in carbon dioxide pressure in the arterial blood.

    3. *Effect of Varying Applied Peak Voltage on the Depth of Inspiration and Minute Volume.* Figure 2 demonstrates the relationship between the intensity of peak voltage and the corresponding minute volume that resulted from the stimulation of a

TABLE I.   COMPARISON OF MINUTE VOLUMES AND PARTIAL PRESSURES OF OXYGEN AND CARBON DIOXIDE OF ARTERIAL BLOOD DURING SPONTANEOUS RESPIRATION AND ELECTRICALLY INDUCED ARTIFICIAL RESPIRATION IN THE ABSENCE OF SPONTANEOUS RESPIRATION

EXP. NO. AND ANIMAL	WT.	ANESTHESIA	SPONTANEOUS RESP.			MEANS OF INHIBITING SPONTANEOUS RESP.	ELECTROPHRENIC RESPIRATION			
			M.V.	pO₂	pCO₂		After onset of EPR	M.V.	pO₂	pCO₂
	$kg.$		$cc.$	$mm.$	$mm.$		$hrs.$	$cc.$	$mm.$	$mm.$
11 Cat	3.0	Chloralose				Procaine into 4th ventricle	1.3	317	118	49
							3.2	254	114	50
							7.4	345	104	48
							21.0	295	115[3]	44[3]
12 Cat	4.3	Chloralose	500	101	47	Procaine into 4th ventricle	1.0	530	101	45
			450	104	45					
13 Dog	12.5	Chloralose	1760	86	47	'Take over' with EPR	1.0	3940[1]	108	30
							1.8	3170[1]	96	31
							2.6	2260[1]	89	45
16 Dog	13.0	Chloralose	2690	83	33	Procaine into 4th ventricle	0.3	3980	87 ·	30
							1.0	3560	89	31
							1.1	3830	86	31
							2.3[2]	7160[2]	100[2]	24[2]
18 Dog		Morphine & urethane	2340	34	72	'Barbiturate' poisoning	0.7	3340	74	51
							1.6[2]	7040[2]	107[2]	20[2]
17 Monkey	2.9	Nembutal	380	102	37	'Take over' with EPR	0.3	550	120	36
							0.5	420	111	43

    [1] Minute volume regulated by adjusting peak voltage.   [2] Both phrenic nerves stimulated.
[3] Single determination.

single phrenic nerve in a cat. Depth of respiration (voltage) alone was varied. The respiratory rate was kept constant throughout. *Experiment 13* in table 1 shows that the arterial oxygen and CO₂ tensions can likewise be regulated by the appropriate adjustment of peak voltage and thus of the minute volume.

    Pneumotachograms (tracheal air flow recordings). Figure 3, *A* through *H*, demonstrates the effect of increasing the intensity of peak voltage upon the air flow pattern in a cat. *A* is the animal's spontaneous respiratory pattern. *B* is the air flow pattern with a peak voltage of 1.84 volts. *C, D, E, F, G* and *H* show the effect of increasing peak voltage. The three factors worthy of note which occur when peak voltage is increased are 1) the increase in the depth of inspiration and air exchange,
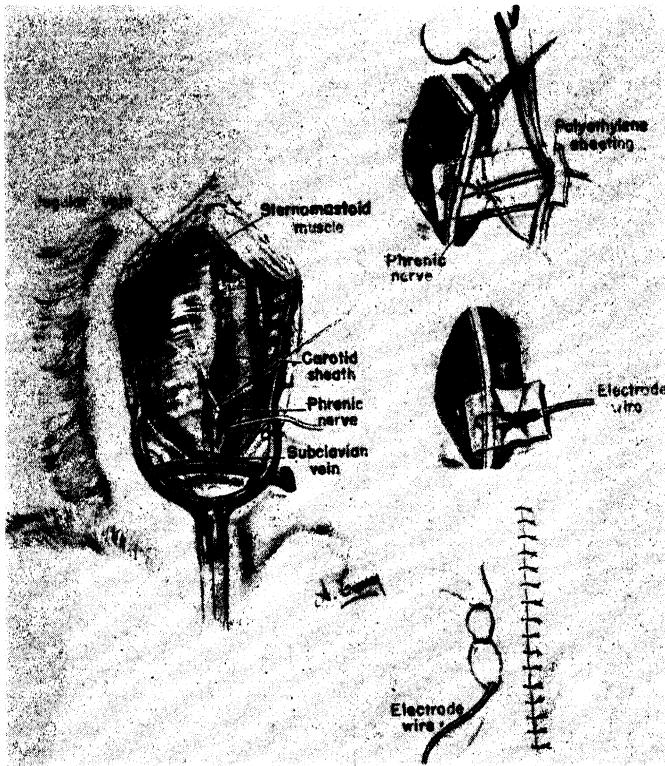
Fig. 1. TECHNIQUE OF ELECTRODE APPLICATION. *A*, position of bared silver wire loop surrounding phrenic trunk in dog. A silk suture firmly secures end of wire to other limb of loop. *B*, thin polyethylene sheeting is then placed under the nerve and folded back to enclose electrodes. A silk suture is introduced in such a way as to secure insulating sheeting firmly to electrode and to itself. *C*, phrenic nerve cannot escape contact with silver-wire electrode but is not compressed by it; electrode cannot stimulate any structure other than the phrenic nerve. *D*, end result. Lead wire emerging from puncture wound; incision is closed.
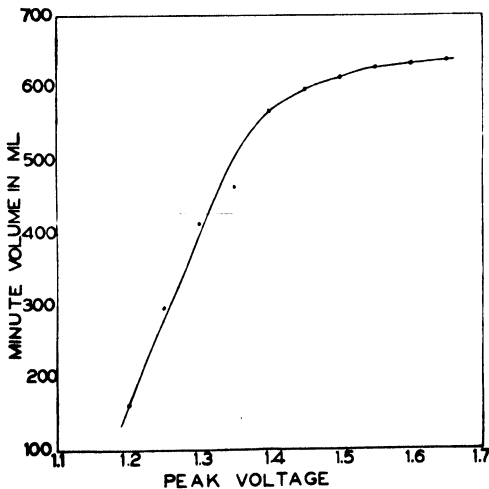
Fig. 2. RIGHT PHRENIC NERVE STIMULATION in cat in absence of spontaneous respiration (intraventricular procaine). Relationship between peak voltage applied to nerve and the corresponding minute volume is demonstrated. Crossing horizontal line indicates average minute volume value for spontaneous respiration in same animal.

2) the increase in the ratio of inspiration to the total respiratory cycle and 3) the increase in the sharpness of inspiratory effort at the higher voltages.

Pneumotachogram of electrophrenic respiration by 'remote' control. **Figure 3** *I* is a tracing from the same animal during electrophrenic respiration by the 'remote' control technique (11, 12) after spontaneous breathing had been abolished by the injection of procaine into the fourth ventricle. The secondary coil was not actually
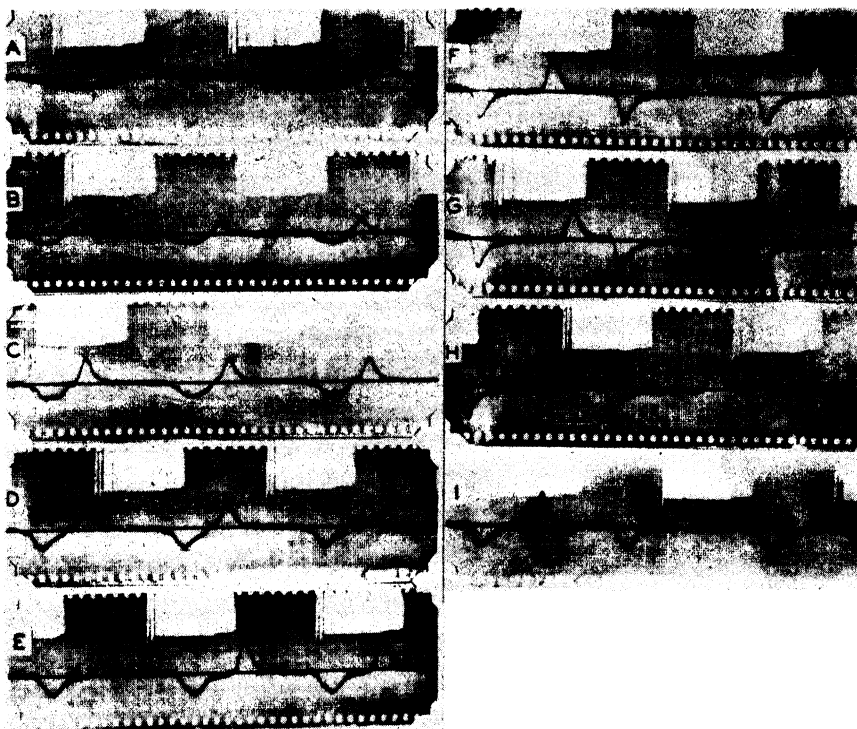
Fig. 3. EFFECTS ON AIR FLOW RECORDINGS of increasing peak voltage applied to right phrenic nerve of cat. *A*, spontaneous respiration. *B*, phrenic stimulation with peak voltage of 1.84. *C*, peak voltage of 1.94 volts. *D*, peak voltage of 2.04. *E*, peak voltage of 2.16. *F*, peak voltage of 2.50 volts. *G*, peak voltage of 4.00. *H*, peak voltage of 10.00. *I*, phrenic stimulation by "remote control" technique. Area below base line indicates inspiration; area above the base line indicates expiration. Area below base line is directly proportional to amount of inspired air.

implanted under the skin but was separated from the primary by a distance of more than one inch.

4. *Prolonged Experiments.* Three prolonged experiments were carried out, using 2 cats and 1 dog. In the first, a cat, spontaneous breathing was abolished by large intravenous doses of sodium nembutal. It was possible to maintain the animal for 12 hours on electrophrenic respiration. Due to the fact that large doses of barbiturate were necessary, the circulation was profoundly depressed and it was felt that this was the cause of the cat's death rather than failure of the neuromuscular mechanism of the diaphragm. The second experiment lasted 16 hours and was probably

terminated by a pulmonary embolus which arose from the dog's femoral vein at the site of multiple venepunctures and an indwelling needle. The third prolonged experiment was performed in a cat and lasted 22 hours. The animal was maintained in excellent condition during this entire period by the electrical stimulation of only the right phrenic nerve while respiration was abolished by the intermittent injection of 1.0 per cent procaine into the fourth ventricle. At the end of 21 hours the partial pressure of O_2 in the blood was 115 mm. Hg and that of CO_2 was 44.5 (single determination, *experiment 11* in table 1). The animal was supported by Ringer's solution given intravenously at intervals throughout the experiment. The experiment was unfortunately terminated by tracheal obstruction in the form of an unsuspected mucous plug in the tracheal cannula. The diaphragm was contracting vigorously at that time.

DISCUSSION

It is apparent from the data in table 1 that adequate aeration, as reflected by minute volume, arterial blood oxygenation and the elimination of carbon dioxide, can be achieved by the submaximal electrical stimulation of a single phrenic nerve in the absence of spontaneous respiratory activity. The data in table 1 and figures 2 and 3 demonstrate that minute volumes, appreciably in excess of those which the animal spontaneously performs to maintain adequate aeration under anesthesia, can be achieved by the use of submaximal stimulation of one phrenic nerve. Maximal stimulation of one phrenic nerve increases ventilation and bilateral phrenic stimulation produces still greater minute volumes.

Table 1, figures 2 and 3 indicate that the tidal volume and minute volume can be regulated satisfactorily by adjusting the voltage applied to the phrenic nerve in such a way as to cause the diaphragm to contract feebly or forcefully as may be desired. In addition, the ratio of the length of inspiration to the length of the total cycle can be varied from 25 to 72 per cent (fig. 3). The sharpness of inspiratory effort can likewise be regulated. Thus a variety of respiratory rates and air flow contours are obtainable.

The prolonged experiments indicate that the neuromuscular mechanism is capable of sustaining prolonged electrical stimulation and the method is thus capable of maintaining life for prolonged periods of time. It is our opinion that in these experiments the failure at the end of the prolonged periods was not due to failure of the neuromuscular mechanism. Under conditions not necessitating deep barbiturate narcosis or central nervous system interference, it is anticipated that stimulation could be successfully maintained for considerably longer periods. The work of Fender (13) is of considerable interest in this connection. He implanted silver electrodes on both splanchnic nerves of the dog and effectively stimulated them with from 6 to 8 volts, 8 hours a day, 6 days a week, for $5\frac{1}{2}$ months. At the end of that time the nerves responded well to stimulation and showed no anatomic evidence of injury.

Experimentally, the method may prove useful in several ways: *a*) production of regular breathing in hemodynamic studies, when irregular breathing often beclouds the interpretation of vascular data, *b*) elucidation of neurogenic control of

breathing, as exemplified by marked differences between this method and the usual positive pressure respiration (to be reported separately), c) separation of ventilation into diaphragmatic and intercostal components, d) separation of respiratory weakness into central and neuromuscular components, and e) ease of obtaining reproducible ventilation patterns in studies of lung absorption of inhaled gases, aerosols etc.

Other observations to be reported elsewhere (14) have demonstrated that, as was anticipated, the human phrenic nerve and diaphragm act in much the same fashion as do those of the experimental animal. The phrenic nerve in man lies directly behind the sternocleidomastoid muscle on the belly of the anterior scalene. Exposure of the nerve can be accomplished in a matter of minutes under local anesthesia. It would be feasible, therefore, to apply this method of artificial respiration to human patients if, eventually, the advantages of the method are established in man.

Although much preliminary work remains to be done, the potential advantages of this method have been considered. These include a) portability and low cost of the apparatus, b) ease of nursing care and freedom of movement of the patient, and c) therapeutic effect of keeping a partially paralyzed diaphragm active. Candidates for this method would be those with intact phrenic nerves, for example patients with bulbar depression or paralysis or high spinal anesthesia. Possible use of the method in patients with anterior horn cell disease must await further study.

Prerequisites to extensive use of the electrophrenic method of artificial respiration in man are a) clear evidence that electrical stimulation of the phrenic does not injure the nerve or its anterior horn cells and b) development of a means of stimulating the phrenic without an operative procedure.

Chaffee and Light (11, 12) have devised a method for the 'remote' stimulation of the nervous system. The method was also used by Fender (13). 'Remote' stimulation is accomplished by imbedding a small secondary coil under the skin with one indifferent electrode and one fine stimulating electrode leading to the nerve that it is desired to stimulate. Later, it is possible to stimulate the nerve in question by bringing the primary coil near the implanted secondary (but external to the animal or patient) and thereby induce a known current in the previously implanted secondary coil. In this way, artificial respiration could be induced without the necessity for continued direct contact between the source of current and the phrenic nerve. This obviates the necessity for having a lead wire emanate from the wound. In *experiment 12* a cat was stimulated by such a 'remote control' technique and figure 3 *I* is the pneumotachographic tracing taken while this type of artificial respiration was being produced. The disadvantage of this technique is the fact that the implanted coil must be of considerable size to insure an adequate stimulus and this disadvantage probably outweighs the gain from avoiding the presence of a single lead wire. For experimental application the most satisfactory method at present is the technique of implanting a single electrode.

<div align="center">SUMMARY</div>

It is feasible to produce artificial respiration by the electrical stimulation of one or both phrenic nerves. By this means, the animal's spontaneous minute volume

can easily be exceeded and the tensions of oxygen and carbon dioxide in arterial blood maintained at satisfactory levels for as long as 22 hours in the absence of spontaneous respiratory activity. The rate and depth of respiration and the contour of respiratory air flow can be readily modified within broad limits.

Clinical and experimental implications of the method have been presented.

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BLOOD SUGAR RESPONSE TO ANOXIA DURING ACCLIMATIZATION

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THERE are several objective physiologic criteria which can be used to determine adaptation to altitude. The most important of these are: an increase in the total ventilation of the lungs; changes in alveolar CO_2 and O_2 tensions; alterations in blood chemistry; an increase in the amount of hemoglobin and number of red blood cells; certain changes in the circulatory system and in gastro-intestinal function.

Acclimatization is an involved process and is not at all well understood. It is desirable that as many criteria as possible be established. On this account it seemed worthwhile to study the effect of acclimatization on blood sugar response to anoxic anoxia.

METHODS

Six dogs were used in these experiments. Throughout the course of the study they were fed an adequate diet including table scraps, purina dog chow and a liberal amount of milk. Before being subjected to test for blood sugar response they were fasted for 24 hours. The ranges of control-blood sugar values in the 6 dogs before exposure to anoxia are shown in table 1.

The animals were subjected to a barometric pressure of 254 mm. of Hg (approximate altitude of 28,000 ft.) in a low-pressure chamber for 15 minutes and blood sugar determinations were then made (Folin-Wu method.) Several such determinations were made at weekly intervals. After the control figure had been established, the animals were exposed to intermittent anoxia of 303 mm. Hg (approximate altitude of 24,000 ft.) 4 hours each day, including Sunday, until they became acclimatized as shown by the fact that exposure to a simulated altitude of 28,000 feet did not cause elevation of the blood sugar.

Daily exposures were then discontinued so that the animals might lose their acclimatization. In order to follow the process of deacclimatization, they were tested by a single exposure to a barometric pressure of 254 mm. Hg for a 15-minute period at varying intervals, but not, as a rule, oftener than once a week.

RESULTS

Five of the 6 animals showed acclimatization as evidenced by the fact that when subjected to a pressure of 254 mm. Hg for 15 minutes, no significant elevation of blood sugar occurred. One animal which was extremely sensitive to anoxia showed only partial acclimatization.

The results of 3 representative animals are shown in the accompanying figures. *Dog 2* (fig. 1) lost most of its acclimatization (as regards response of blood sugar to anoxia) within a period of about 15 days. On the other hand *dog 4* (fig. 2) started losing its acclimatization after about 6 weeks, but lost it slowly and did not deacclimatize until about 5 months had elapsed. *Dog 5* (fig. 3) still showed acclimatization 7 months after it had been exposed to intermittent anoxia; further determinations were not made on this animal. The data obtained from the 2 remaining dogs were

TABLE 1. RANGE OF CONTROL BLOOD SUGARS ON DOGS BEFORE EXPOSURE TO ANOXIA

DOG NO.	MG/PER 100 ML.	NO. OF DETERMINATIONS
2	91–110	9
3	87–111	9
4	108–128	9
5	105–124	9
6	107–122	10
8	101–105	2
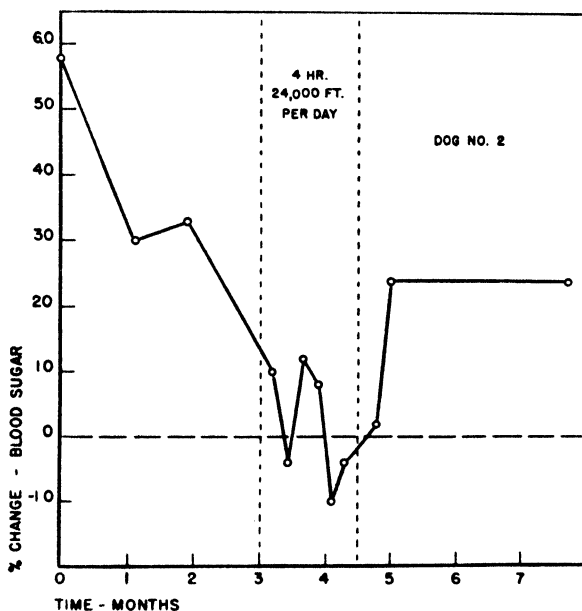
Fig. 1. ACCLIMATIZATION of the hyperglycemic response to 28,000 ft.

as follows: the results in one of the dogs were indeterminate at the end of 2 months; the other animal had hardly lost any of his acclimatization at the end of 2 months.

DISCUSSION

It is of interest that it took only a relatively short period of time (about a week) to acclimatize these animals, as far as the blood sugar response to anoxia was concerned. In work reported from this laboratory (1) on the effect of intermittent anoxia on the hemoglobin and on the number of red blood cells, it was found that there was no significant rise in either until nearly three weeks had elapsed. This

same time element was found also in experiments we reported concerning acclimatization and gastric emptying (2).   These experiments were also performed on dogs.

In order to demonstrate that there is virtually an acclimatization for every altitude and to show that the adrenal glands were not exhausted, the acclimatized animals were subjected to a pressure of 208 mm. Hg (approximate altitude of 32,000
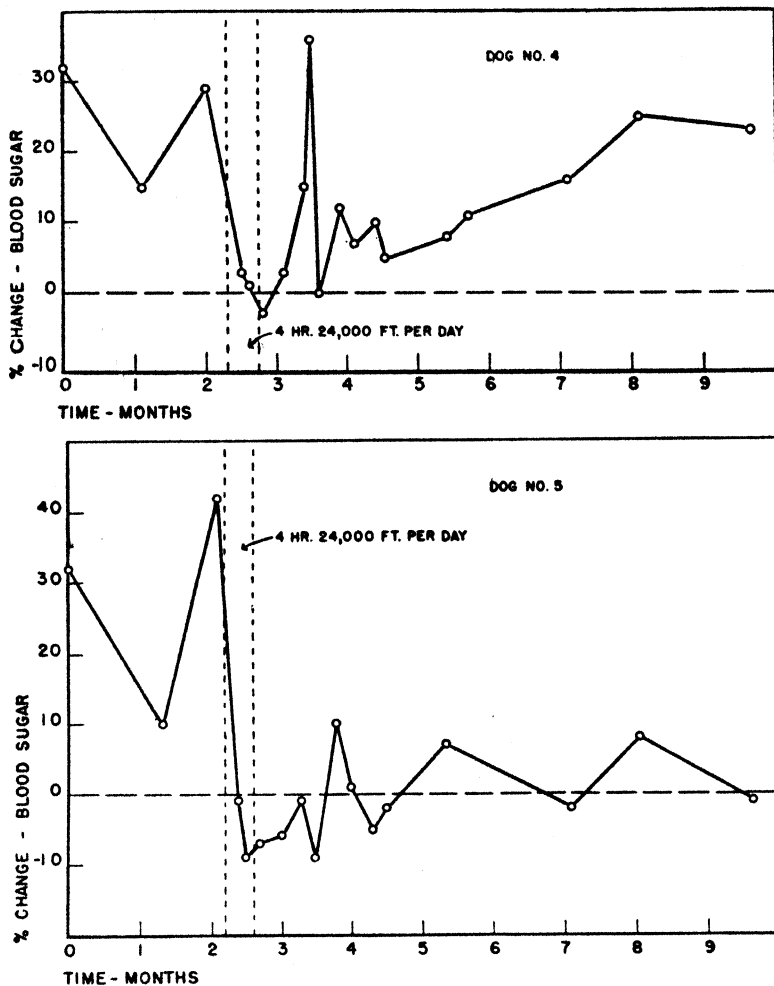
Figs. 2 (*upper*) and 3 (*lower*). ACCLIMATIZATION of the hyperglycemic response to 28,000 ft.

ft. for 15 minutes).   Five of the 6 dogs showed a significant rise in blood sugar. However, the animal which showed only partial acclimatization died within a few minutes after being exposed to this simulated extreme altitude.

It is noteworthy that 2 of the surviving animals did not deacclimatize—at least during the time they were under observation.   It is difficult to account for this

phenomenon.   There are several factors which might be mentioned: *a*) anoxia disturbed the balance between the vago-insulin and the sympathico-adrenal systems; *b*) anoxia impaired the function of the liver depots so that the sugar was not released; *c*) anoxia changed the threshold of the response of blood sugar to altitude.   All of these factors are more or less speculative and, as far as we are aware, no definite proof for any of them exists.

If the vago-insulin and the sympathico-adrenal balance were disturbed by the anoxia, it is difficult to see why these mechanisms apparently were still in balance when the animals were subjected to more severe degrees of anoxia than those to which they had been acclimatized.   Relative to the effect of acclimatization on the liver, Gellhorn and Packer (3) working with unanesthetized rabbits reported that brief periods of anoxia tend to antagonize the effect of insulin and cause a quicker recovery of the blood sugar to normal values.   Prolonged anoxia, however, leads to an aggravation of the hypoglycemia and a progressive fall of the blood sugar curve. These authors feel that the failure of prolonged anoxia to bring about a recovery of the blood sugar curve is due to the inability of epinephrine to liberate glucose from the liver.   The question arises whether this could be applied to acclimatized animals. Lastly, anoxia may change the threshold of the response of blood sugar to altitude by some mechanism not as yet understood.   It would not necessarily have to be central in character.

It is hard to believe that those animals which did not deacclimatize were permanently acclimatized as far as the blood sugar response to anoxia was concerned. It is known that animals upon return to sea-level lose their characteristic physiologic changes associated with high altitude adaptation.   For example, when acclimatized animals are brought down to sea-level, the number of red blood cells and the amount of hemoglobin return to normal values within a relatively short time.   It is quite likely that some organs or body systems retain their acclimatization longer than do others, but it does not seem likely that they would retain it indefinitely.

It should be mentioned that subjectively the animals looked more at ease at altitude after they had been acclimatized than they did at the beginning of the experiment.   Their muscular coordination, too, upon removal from the low-pressure chamber was much better.   The animals did not lose any appreciable amount of weight in the course of the experiments and, save the one which died, remained healthy and vigorous throughout.   There was no increase in the amount of hemoglobin in the 4 dogs which showed acclimatization (in response of blood sugar to anoxia) within a period of about a week.   Two animals, however, which were subjected to intermittent anoxia for a longer period showed, as would be expected, an appreciable rise in the amount of hemoglobin.

SUMMARY

After the normal response of blood sugar to anoxia (254 mm. Hg) was determined in 6 dogs, the animals were subjected to a pressure of 303 mm. Hg for 4 hours a day.   Five of the 6 animals became acclimatized as far as the blood sugar response was concerned as evidenced by the fact that there was no increase when exposed to

a pressure of 254 mm. Hg.　Several animals deacclimatized within a relatively short time, but one animal showed considerable acclimatization at the end of 2 months, and another animal apparently was still acclimatized at the end of a 7-month period.

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EFFECT OF MUSCLE WORK UPON TOLERANCE OF EVISCERATED RAT FOR GLUCOSE

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IT HAS been shown (1) that muscular work accelerates the rate of fall of blood glucose in eviscerated and eviscerated-nephrectomized rats. The present experiments demonstrate the marked effect of muscle work upon the glucose load which the eviscerated rat can tolerate in the presence and absence of insulin.

METHODS

Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At a weight of 185 to 205 grams, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 ($\pm$2) grams they were anesthetized (intraperitoneal injection of 18 mg. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure of Ingle and Griffith (2). Hemostasis was attained by applying a gelatin sponge (Gelfoam Upjohn) saturated with a solution of thrombin to the stumps of the esophagus, colon, ligated vessels and between the muscle and skin when the incisions were closed.

Immediately following the operation, the animals were prepared for the stimulation of muscle according to Ingle (3) and with the following modifications. A Nerve Stimulator (Model B, Upjohn) was used to stimulate muscle at the rate of 5 times per second. The duration of each pulse was 20 msec. and the intensity was 20 ma. In *experiment 1*, the stimulus was applied to the left leg only; in *experiment 2*, the stimulus passed from the right back foot to the contralateral back foot thereby activating the entire musculature of both legs. The work of the left gastrocnemius was registered on automatic work recorders. Each recorder revolution represented approximately 400-gram centimeters of work. The animals were enclosed in a cabinet with temperature constant at 26.5° ($\pm$.5) C.

Solutions of glucose (C.P. Dextrose, Merck) with and without insulin (crystalline zinc, Lilly) were infused into the jugular vein at a constant rate by means of a continuous injection machine which delivered fluid from each of 12 syringes at the rate of 20 cc. in 24 hours. The glucose load is expressed as milligrams of glucose per 100 grams of rat per hour (mg/100/h.). Insulin was given at the rate of 4 U per 24 hours. The infusions covered a period of 2 hours.

Analyses of glucose were made on tail blood by the method of Miller and Van Slyke (4).

EXPERIMENTS AND RESULTS

Experiment 1 (fig. 1) was a study of the effect of stimulating the gastrocnemius muscle of one leg upon the tolerance of the eviscerated rat for glucose. The following

glucose loads were covered: 0, 16, 24, 36, 44, 48, 52, 70, 100, 110, 120 and 130/100/h. The following experimental conditions with 8 rats per group were represented: no work, no insulin; work, no insulin; no work, insulin; and work, insulin. During the 2-hour period the glucose load tolerated by the non-working rat without insulin was approximately 16/100/h.; with insulin the value was increased to 72/100/h. Under similar conditions the stimulation of one leg in rats without insulin increased the tolerance for glucose to approximately 48/100/h.; with insulin the tolerance was raised to approximately 110/100/h.

Experiment 2 (fig. 2) was a study of the effect of activating all of the musculature of both back legs upon the tolerance of the eviscerated rat for glucose. The following glucose loads were covered: 50, 72, 100, 120, 140, 160, 180, 200, 220, 240, 260 and 280/100 h. Working rats were studied with and without insulin. The average
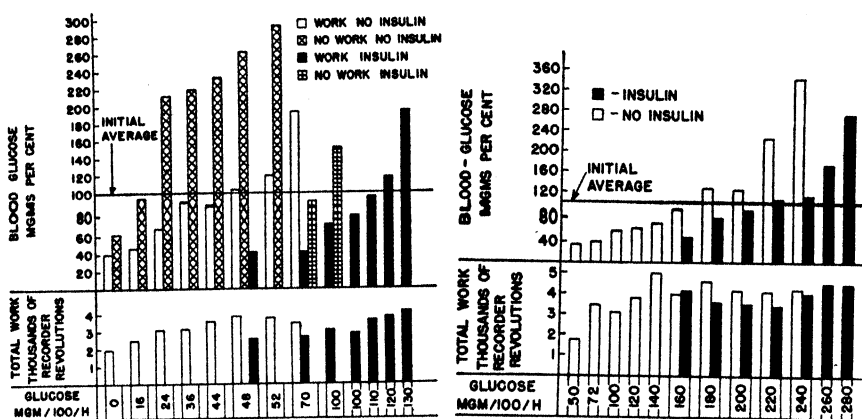
Fig. 1 (*left*). EFFECT OF STIMULATING THE GASTROCNEMIUS MUSCLE of one leg upon the glucose tolerance of eviscerated rats with and without insulin. Average level of blood glucose at the end of 2 hours. Eight rats per group.

Fig. 2 (*right*). GLUCOSE TOLERANCE OF EVISCERATED RATS subjected to stimulation of both back legs with and without insulin. Average level of blood glucose at the end of 2 hours. Eight rats per group.

glucose tolerance for rats without insulin was approximately 160/100/h.; with insulin this value was increased to approximately 220/100/h.

As shown in figures 1 and 2 the amounts of work recorded during 2 hours of stimulation were roughly proportional to the glucose load in those animals which did not receive insulin. When the level of blood glucose was suppressed by the administration of insulin the work performance was definitely depressed. There was no tendency for the addition of insulin to improve work performance under these experimental conditions.

DISCUSSION

Under resting conditions the tolerance of the eviscerated rat for glucose is very low, although it can be increased somewhat by insulin. It is probable that this low requirement for glucose is due in major part to inactivity. During muscular work

the requirement for glucose can be increased ten-fold without the addition of insulin. We have not determined the fate of the glucose or the changes in carbohydrate stores in the peripheral tissues. It seems reasonable to draw the tentative conclusion that the stimulation of muscle causes a marked increase in the utilization of glucose without any change in the insulin content of the body fluids. It is also reasonable to suggest that these rats were insulin-deficient following the removal of the pancreas by evisceration.

The addition of insulin increased the tolerance for glucose by approximately the same load (60/100/h.) in the resting animal, in the animal subjected to stimulation of the gastrocnemius of one leg and in animals subjected to stimulation of both hind legs. This may have been fortuitous. The administration of insulin did not improve work performance. On the contrary, when the level of blood glucose was lowered by insulin the performance of work was somewhat depressed. It is possible that the action of insulin favored pathways of glucose conversion which competed with the contracting muscle for the glucose.

The effect of work upon the carbohydrate requirement of the eviscerated rat should be explored further in animals made severely diabetic prior to evisceration. A full interpretation of the results would require tracing the pathways of carbohydrate utilization during rest and work.

SUMMARY

The effect of work upon the tolerance of the eviscerated rat for intravenously administered glucose was studied during a period of 2 hours. In *experiment 1*, the gastrocnemius muscle of one leg was stimulated to lift 100 grams at the rate of five times per second. The average glucose load tolerated by the non-working rat without insulin was approximately 16/100/h.; with insulin the value was 72/100/h. Under similar conditions the stimulation of one leg in rats without insulin increased the tolerance for glucose to approximately 48/100/h.; with insulin the approximate value was 110/100/h.

In *experiment 2*, the stimulus passed from one back foot to the contralateral back foot thereby activating the entire musculature of both hind legs. The average value for glucose tolerance without insulin was approximately 160/100/h.; with insulin the approximate value was 220/100/h.

Work performance was roughly proportional to the glucose load in those animals which did not receive insulin. The administration of insulin suppressed work performance at the lower glucose loads. In no instance did the administration of insulin enhance the ability of the eviscerated rat to work.

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EFFECTS OF THE PITUITARY GROWTH AND ADRENOCOR-TICOTROPIC HORMONES ON THE URINARY GLUCOSE, NITROGEN AND KETONE BODIES OF DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET[1]

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PREVIOUS work from this laboratory has demonstrated that the administration of pure adrenocorticotropic hormone enhanced both the glycosuria and urinary nitrogen excretion of diabetic rats which were maintained on a stock diet (1). The hypophyseal growth hormone produced nitrogen retention but had little effect upon the glycosuria of such animals. Either adrenocorticotropic hormone or growth hormone increased the ketonemia and ketonuria of fasted normal rats (2). Adrenocorticotropic hormone increased both the glycosuria and urinary nitrogen excretion of diabetic rats which were maintained on a carbohydrate-free diet (3). The effect of growth hormone under these conditions has not been investigated previously. It is the purpose of this paper to present some experiments in which these two hypophyseal hormones were administered to diabetic rats, which were maintained on a carbohydrate-free diet, and the resulting changes in the urinary excretion of glucose, ketone bodies and nitrogen were investigated. The experiments with adrenocorticotropic hormone are in addition to those previously reported (3).

METHODS

All animals used were male rats of the Long-Evans strain between 55 to 65 days of age at the time of production of diabetes. Diabetes was produced by the intraperitoneal injection of 200 mg./kg. body weight of alloxan monohydrate (Eastman) on each of two successive days. Ten rats which had a persistent and uniform glycosuria while on the carbohydrate-free diet[2] were selected for study. Although the degree of glycosuria was uniform from day to day in each animal, it dif-

[2] The carbohydrate-free diet consisted of alcohol-extracted casein 71%, hydrogenated vegetable oil (Crisco) 24%, salts No. 4 (6) 4% and liver syrup and B vitamin mixture one %. This furnished the following amounts of vitamins/kg. diet: 2-methyl-1,4-naphthoquinone 5 mg., thiamine HCl 5 mg., riboflavin 10 mg., pyridoxine HCl 5 mg., *p*-aminobenzoic acid 10 mg., nicotinic acid 20 mg., calcium pantothenate 50 mg., inositol 400 mg., pteroylglutamic acid 5.5 mg., synthetic *d*-biotin 0.3 mg. and choline chloride one gm. One cc. of a fat-soluble vitamin mixture was fed at about the mid-point of the metabolic study period. This consisted of 6 mg. α-tocopherol (Merck), 115 chick units vitamin D and 800 USP units vitamin A in 650 mg. corn oil (Mazola).

fered from one animal to another, varying from 220 mg. to 3980 mg/day. Thus, the effect of the hormones could be evaluated in animals with varying degrees of severity of diabetes.

By trial and error it was determined how much food would be consumed completely by each animal each day and this amount was fed daily throughout the experiment. Therefore, with but one exception, the food intake was constant for each animal throughout the entire period of observation. One rat during the injection of adrenocorticotropic hormone failed to eat as much as it had during the pre-injection control period. During the post-injection control period it was fed daily an amount of food equal to the average daily consumption during the injection period.

The animals were kept in individual metabolism cages and were fed at the same time each morning. Urines were collected through large ribbed glass funnels into wide-mouthed Erlenmeyer flasks which contained a layer of toluene as a preservative. Feces were separated from the urine by perforated porcelain discs. Collections were made each morning by washing down the funnels with 300 to 400 cc. of distilled water.

Glucose was determined by the Somogyi (4) method and is reported as total reducing substances. Correction for non-fermentable reducing substances were not carried out, since studies with yeast fermentation have shown that the urinary content of non-fermentable reducing substances is as negligible in animals upon this diet as it is in animals upon the stock diet (1). Urinary nitrogen was determined by the micro-Kjeldahl procedure. In these experiments fecal nitrogen analyses were not done. Previous unpublished observations have confirmed that the fecal nitrogen excretion is constant as long as the dietary intake is constant and, in addition, have shown that growth hormone or adrenocorticotropic hormone administration is without effect upon the fecal nitrogen excretion. Thus, changes in urinary nitrogen excretion may be taken as a valid index of changes in nitrogen balance under the condition employed in these experiments. Urinary ketone bodies were determined by the method of Van Slyke (5) with Denige's reagent. In two of the experiments with growth hormone ketone bodies were not determined (*Exp. 5 and 6*).

The growth hormone and adrenocorticotropic hormone[3] used were pr pared according to the previously published method (7, 8) and were administered intraperitoneally at a dose level of 3 mg/day, three injections of one mg. each being given at intervals of about five hours during the day.

Before administering a hormone, the urinary excretion of glucose, nitrogen and ketone bodies was determined for a pre-injection control period of from 8 to 18 days. The duration of the period of hormone administration in most instances was five days. In some cases, after the administration of one hormone for a five-day period, injection of the other hormone was started immediately without an intervening control period. In all cases following the cessation of injection a post-injection control period of from five to nine days was carried out, the animals being followed for the longer period when the degree of glycosuria and urinary nitrogen excretion did not immediately return to the pre-injection level.

RESULTS AND COMMENT

The data of the experiments are presented in tables 1, 2 and 3 and in figures 1 and 2. Figure 2 contains data in addition to that incorporated in the tables and figure 1 shows the type of day-by-day variations that were encountered. In the graphic presentation, individual daily values representing the response of 2 individual rats to each hormone are shown. In the tabular presentation, the average excretion during the injection period has been compared statistically with the average excretion during the control period. A p value of 0.05 or less was considered to be significant. Only the average of the pre-injection control period is shown in the table except when the pre- and post-injection control periods were different, in which case the mean of each is given. There is one exception. In the case of *experiment 9*, only the post-injection control data were presented. This is the animal that reduced its food intake during the injection period.

Inspection of table 1 shows that the administration of growth hormone did not

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[3] The authors are indebted to Doctor Choh Hao Li for the preparation of the hormones.

produce a significant increase in glycosuria in any of the 6 animals. Figure 2 demonstrates that, even when the glycosuria had been enhanced by prior administration of adrenocorticotropic hormone, growth hormone did not maintain the glycosuria at the

TABLE 1. EFFECT OF GH AND OF ACTH ON THE URINARY EXCRETION OF GLUCOSE BY DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET

EXP. NO.	GM. DIET/DAY	HORMONE/DAY IN MG.	URINARY GLUCOSE IN MG/DAY			
			Control period	Injection period	Change	p[4]
1	10	3 GH	220 ± 30[3] (8)[2]	320 ± 80 (5)	+100	0.20
2	14	3 GH	480 ± 110 (8)	820 ± 150 (5)	+340	0.07
3	12	3 GH[1]	2040 ± 90 (9)	2280 ± 100 (5)	+240	0.10
4	12	3 GH[1]	2930 ± 180 (7)	3020 ± 180 (5)	+90	0.30
5	16	3 GH	3300 ± 100 (17)	3330 ± 200 (6)	+30	0.85
6	16	3 GH	3660 ± 70 (17)	3780 ± 150 (6)	+120	0.40
8	12	3 ACTH[1]	930 ± 70 (8)	2930 ± 150 (5)	+2000	<0.01
9	7.6	3 ACTH[1]	1770 ± 140 (9)	2470 ± 230 (5)	+700	0.01
10	10	3 ACTH	230 ± 40 (9)	760 ± 140 (5)	+530	<0.01
11	14	3 ACTH	560 ± 120 (9)	2040 ± 210 (5)	+1480	<0.01
12	12	3 ACTH	2040 ± 90 (9)	2010 ± 190 (5)	-30	0.90
			1390 ± 210 (4)		+620	00.04

[1] First day only 2 mg.   [2] Number of days of observation.   [3] Standard deviation of the mean
[4] From Fisher's (9) table of *t*.

TABLE 2. EFFECT OF GH AND OF ACTH ON THE URINARY EXCRETION OF NITROGEN BY DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET

EXP. NO.	GM. DIET/DAY	HORMONE/DAY IN MG.	URINARY NITROGEN IN MG/DAY			
			Control period	Injection period	Change	p[4]
1	10	3 GH	780 ± 15[3] (8)[1]	684 ± 15 (5)	-96	<0.01
2	14	3 GH	1062 ± 15 (8)	945 ± 19 (5)	-117	<0.01
3	12	3 GH[2]	948 ± 21 (9)	849 ± 16 (5)	-99	<.01
4	12	3 GH[2]	891 ± 18 (7)	874 ± 41 (5)	-17	0.65
			1041 ± 35 (5)		-167	<0.01
5	16	3 GH	1369 ± 16 (18)	1152 ± 39 (6)	-217	<0.01
6	16	3 GH	1297 ± 17 (18)	1209 ± 29 (6)	-88	0.02
8	12	3 ACTH[2]	876 ± 17 (8)	1058 ± 18 (5)	+182	<0.01
9	7.6	3 ACTH[2]	612 ± 25 (9)	745 ± 61 (5)	+133	0.02
10	10	3 ACTH	800 ± 17 (9)	870 ± 19 (5)	+70	0.02
11	14	3 ACTH	1074 ± 19 (9)	1207 ± 22 (5)	+133	<0.01
			1183 ± 30 (6)		+24	0.50
12	12	3 ACTH	948 ± 21 (9)	1021 ± 36 (5)	+73	0.06

[1] Number of days observation.   [2] First day only 2 mg.   [3] Standard deviation of the mean.
[4] From Fisher's (9) table of *t*.

higher level. On the other hand, adrenocorticotropic hormone produced a significant increase in glycosuria in 4 of the 5 animals to which it was administered. It is of interest to note that in *experiment 12*, in which there was no increase when compared to the pre-injection control period, the diabetes was of considerable severity,

the rat initially excreting more than 2 gm. of glucose per day in spite of the absence of any dietary carbohydrate.   This observation is in conformity with those previously

TABLE 3.   EFFECT OF GH AND OF ACTH ON THE URINARY EXCRETION OF KETONE BODIES OF DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET

| EXP. NO. | GM. DIET/ DAY | HORMONE/DAY IN MG. | URINARY KETONE BODIES IN MG/DAY | | | |
			Control period	Injection period	Change	p[5]
1	10	3 GH	4.1 ± .82[2] (7)[1]	2.3 ± .70 (5)	−1.8	0.12
2	14	3 GH	4.7 ± 1.13 (7)	5.0 ± 1.94 (5)	+0.3	0.90
3	12	3 GH[3]	3.8 ± .64 (8)	15.8 ± 6.65 (5)	+12.0	0.03
4	12	3 GH[3]	3.9 ± .82 (7)	111.1 ± 28.6 (5)	+107.2	<0.01
7	12	3 GH	3.3 ± .81 (7)	43.1 ± 23.0 (4)	+39.8	0.02
8	12	3 ACTH[3]	3.0 ± .66 (8)	22.7 ± 13.6 (5)	+19.7	0.05
9	7.6	3 ACTH[3]	5.9 ± 3.63 (8)	67.6 ± 15.7 (5)	+61.7	<.01
10	10	3 ACTH	3.7 ± .84 (8)	3.1 ± 1.10 (5)	−0.6	0.65
11	14	3 ACTH	4.6 ± 1.00 (8)	4.9 ± .83 (5)	+0.3	0.80
			2.1 ± .40 (7)		+2.8	<0.01
12	12	3 ACTH	1.6 ± .68 (5)	3.1 ± 1.78 (5)	+1.5	0.40
13	14	3 ACTH[3]	49.5 ± 18.8 (7)	254.0[4] ± 23.4 (4)	+204.5	<0.01

[1] Number of days observation.   [2] Standard deviation of the mean.   [3] First day only 2 mg.
[4] Food consumption reduced to 9 gm. per day during the injection period.   Died at end of 5th day of injection.   Glucose and nitrogen data not presented.

[5] From Fisher's (9) table of *t*.
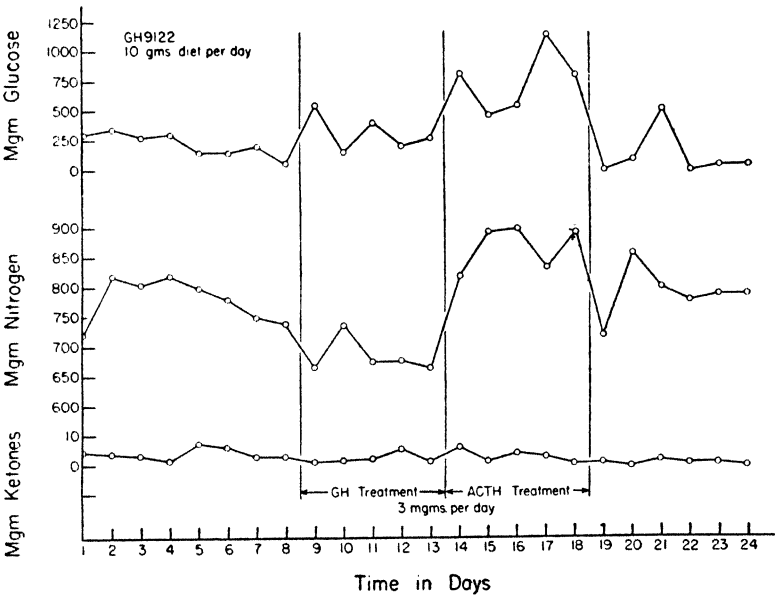
Fig. 1. Effect of GH and ACTH upon the urinary glucose, nitrogen and ketone bodies of a diabetic rat maintained on a carbohydrate-free diet.

reported (3), that the effect of adrenocorticotropic hormone upon the glycosuria under these dietary conditions is less marked when the diabetes is severe.   Also, in this

animal the adrenocorticotropic hormone administration followed the period of growth hormone administration.

The data presented in table 2 show that in each instance in which growth hormone was administered there was a reduction in the excretion of urinary nitrogen. This reduction was significant in six of the seven cases in which statistical comparison was made.   Figure 2 shows a similar effect of growth hormone in the rat that had its nitrogen excretion enhanced by prior administration of adrenocorticotropic hormone. In each instance in which adrenocorticotropic hormone was given there was an increase in urinary nitrogen excretion which was significant in at least one comparison
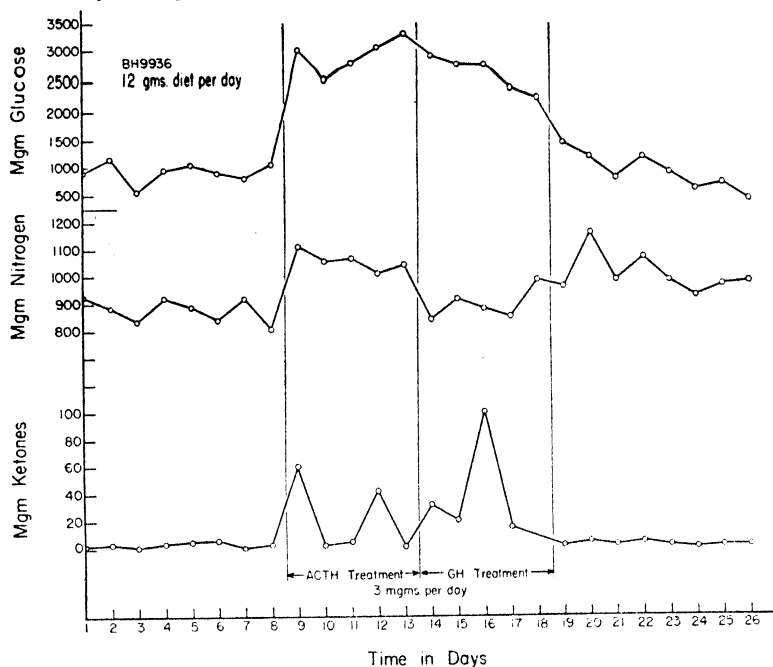
Fig. 2. Effect of GH and ACTH upon the urinary glucose, nitrogen and ketone bodies of a diabetic rat maintained on a carbohydrate-free diet.

in 4 of the 5 animals.   It is clear, therefore, that under these experimental conditions the effect of adrenocorticotropic hormone is to increase both the urinary nitrogen excretion and the glycosuria.   On the other hand, there seems to be little doubt that growth hormone promotes nitrogen retention and is without effect upon the glycosuria.

Both hormones increased the ketonuria of the rats that were severely diabetic. The statistical summary is presented in table 3 and the variable nature of the response is shown in figures 1 and 2.   Growth hormone produced an increase in ketonuria in three of the five experiments.   Using the experiment numbers, cross reference to tables 1 and 2 shows that the increase in ketonuria occurred in those animals that had a marked glycosuria and D/N ratios above 2.69 during the injection period, rather than in the mildly diabetic rats.   Adrenocorticotropic hormone produced an

increase in ketonuria in four of six experiments. Again, this occurred only in animals with a marked glycosuria and high D/N ratios. Since both adrenocorticotropic hormone and growth hormone will increase the ketonuria of fasted normal rats (2), it is not surprising that they have a similar effect in diabetic rats maintained on a carbohydrate-free diet. However, the above data would seem to indicate that the increased ketonuria is present only if the animal is severely diabetic.

CONCLUSIONS

Adrenocorticotropic hormone enhances both the glycosuria and urinary nitrogen excretion of diabetic rats maintained on a carbohydrate-free diet. Growth hormone produces nitrogen retention but does not significantly alter the glycosuria of diabetic rats maintained on a carbohydrate-free diet. If the diabetes is severe, both growth hormone and adrenocorticotropic hormone increase the ketonuria of diabetic rats maintained on a carbohydrate-free diet.

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EFFECTS OF THE PITUITARY GROWTH AND ADRENOCORTI-COTROPIC HORMONES ON THE URINARY GLUCOSE AND NITROGEN OF HYPOPHYSECTOMIZED DIABETIC RATS[1]

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T HIS paper is the last of a series reporting studies of the metabolic effects of the pituitary growth and adrenocorticotropic hormones in diabetic rats. Previous papers have reported experiments involving the administration of these hormones to diabetic rats maintained on a stock diet (1) and on a carbohydrate-free diet (2, 3).

It is the purpose of the present paper to report effects produced in hypophysectomized-diabetic rats by the pure growth and adrenocorticotropic hormones.

METHODS

The hormones used[2] were prepared according to the previously published methods (4, 5). The age and sex of animals used, the method of production of diabetes and the analytical methods for the determination of the daily urinary excretion of glucose and nitrogen were the same as those previously described (1). Animals with a persistent, stable degree of glycosuria were selected for study and were kept in individual metabolism cages.

Two different experiments were carried out: the first, an investigation of the effects of adrenocorticotropic hormone; the second, an investigation of the effects of growth hormone. In each experiment there was a group of hypophysectomized-diabetic rats and a control group of intact diabetic rats. The control group in each case was pair-fed[3] with the corresponding experimental group. The food consumption was restricted prior to hypophysectomy; after hypophysectomy the hypophysectomized rats were allowed to eat *ad libitum*. In each group there were 4 or 5 animals that were carried through the entire period of 26 days of observation. The original number of animals in the groups were larger, but deaths after hypophysectomy, incomplete hypophysectomy and failure to maintain a stable daily food intake reduced the number of animals.

For seven days prior to hypophysectomy the daily urinary excretion of glucose and nitrogen was determined for a pre-hypophysectomy control period. After hypophysectomy the same determinations were made for a seven-day posthypophysectomy control period. The hormones were given for a period of four days and were administered intraperitoneally at a dose of one mg. three times a day. Following the period of hormone administration a post-injection control period of from five to six days was carried out.

Fig. 1. DAILY FOOD CONSUMPTION, urinary nitrogen and urinary glucose of hypophysectomized diabetic rats treated with adrenocorticotropic hormone.
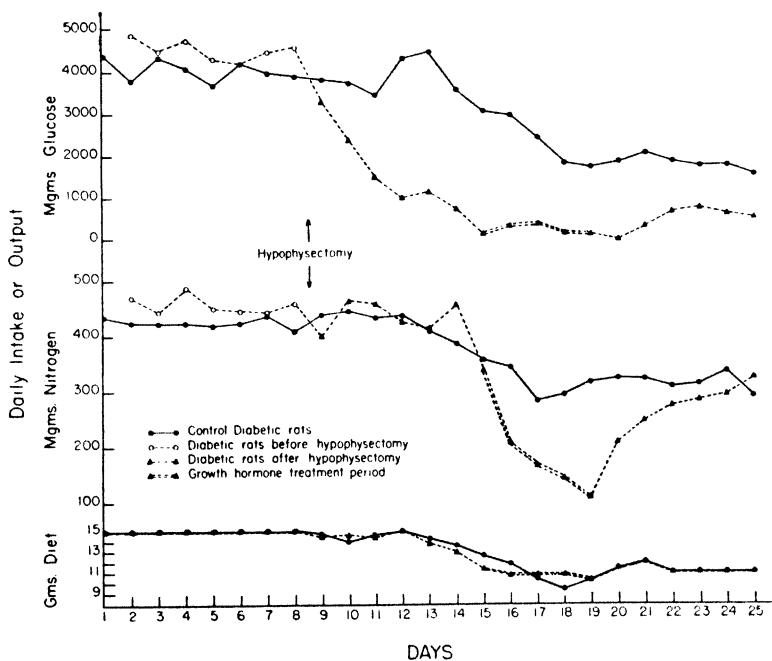
Fig. 2. DAILY FOOD CONSUMPTION, urinary nitrogen and urinary glucose of hypophysectomized diabetic rats treated with growth hormone.

RESULTS AND COMMENT

The data from the experiments are presented in figures 1 and 2 and in table 1. In the graphic presentation is shown the average daily food consumption and urinary excretion of nitrogen and glucose by the group. In table 1 the average of individual hypophysectomized-diabetic rats during the injection period is compared with a control period. Inspection of figures 1 and 2 shows that prior to hypophysectomy the food consumption was quite constant and nearly identical in both the experimental and control groups and that there was essentially no difference in the urinary glucose and nitrogen excretion of the experimental group as compared to the control.

TABLE 1. EFFECT OF 3 MG. PER DAY OF ADRENOCORTICOTROPIC HORMONE AND GROWTH HORMONE ON THE URINARY GLUCOSE AND NITROGEN OF HYPOPHYSECTOMIZED-DIABETIC RATS

HORMONE GIVEN	FOOD INTAKE		URINARY GLUCOSE				URINARY NITROGEN			
	Control period[1]	Injection period	Control period	Injection period	Change	p	Control period	Injection period	Change	p[2]
	gm/day	gm/day	mg/day	mg/day	mg/day		mg/day	mg/day	mg/day	
ACTH	10.0	9.9	1460 ± 190[3]	3010 ± 770	+1550	.03	273 ± 7	330 ± 11	+57	<.01
ACTH	8.7	10.8	930 ± 190	3390 ± 920	+2460	<.01	262 ± 31	356 ± 28	+106	.02
ACTH	10.6	10.7	2990 ± 450	4550 ± 320	+1560	.015	304 ± 24	333 ± 22	+29	.30
ACTH	13.3	14.9	2310 ± 370	4530 ± 630	+2220	<.01	360 ± 15	423 ± 26	+83	<.01
ACTH	13.1	13.2	2860 ± 320	4890 ± 890	+2030	.02	389 ± 31	400 ± 40	+11	.90
GH	11.2	12.1	380 ± 100	660 ± 160	+280	.10	312 ± 17	218 ± 36	−94	.02
GH	11.2	9.5	430 ± 115	220 ± 85	−210	.15	271 ± 22	154 ± 28	−117	<.01
GH	11.2	11.3	120 ± 50	70 ± 20	−50	.30	271 ± 14	160 ± 30	−111	<.01
GH	11.2	10.0	1610 ± 190	120 ± 30	−1490	<.01	297 ± 18	102 ± 8	−195	<.01

[1] For the ACTH-treated animals the last 5 days of the post-hypophysectomy control period was used. For the GH-treated animals the last 5 days of the post-injection control period was used. These control periods were taken as they gave the most uniform food intake compared with the injection period.

[2] From Fisher's table of *t*. A value of 0.05 or less was considered significant.

[3] Standard deviation of the mean.

After hypophysectomy, the hypophysectomized rats exhibited a marked reduction in glycosuria which was greater than could be accounted for by the reduced food intake, since a comparable reduction was not shown by the controls. The urinary nitrogen excretion[4] of both experimental and control groups was reduced slightly and to a comparable degree, seemingly in proportion to the reduction in food intake.

Adrenocorticotropic hormone was administered both to the hypophysectomized-diabetic rats and to their unhypophysectomized controls. In both groups and in all animals there was a striking and significant increase in glycosuria, reaching its peak on the last day of injection. The increase in glycosuria could not be accounted for by an increase in food intake. In both groups there was a definite but not as strik-

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[4] Unpublished observations of fecal nitrogen content have shown that it is not influenced by hypophysectomy nor by growth or adrenocorticotropic hormone administration. Thus, they are not reported in these experiments.

ing or consistent an increase in urinary nitrogen. Thus, previous work regarding the diabetes-enhancing effect of crude adrenocorticotropic preparations (6) has been fully confirmed with the pure hormone. It increases both the glycosuria and urinary nitrogen excretion of diabetic rats. Adrenocorticotropic hormone may also be said to be diabetes-enhancing with respect to the ketonuria of diabetic animals (3), since under appropriate experimental conditions it is increased as well. In these experiments as in the previous ones in which animals were maintained on the stock diet (1), the increased glucose in the urine could not have been derived from the additional protein broken down under the influence of the hormone.

Growth hormone was administered only to the hypophysectomized-diabetic rats, not to their controls. In no case was there produced a significant increase in glycosuria. In fact, in three cases the glycosuria decreased. In every case growth hormone produced nitrogen retention, with its effect apparently persisting for at least 24 hours after the cessation of its injection. Thus, in no sense can growth hormone be considered diabetes-enhancing with respect to its effect upon the protein metabolism of diabetic rats. It is doubtful if growth hormone can be considered diabetes-enhancing with respect to its effect upon the glycosuria of diabetic rats since it was without effect in the present experiment; it was without effect in diabetic rats maintained on a carbohydrate-free high-protein diet (3) and it only occasionally increased the glycosuria of rats on the stock diet (1). However, growth hormone may be said to be diabetes-enhancing with respect to the ketonuria of diabetic rats, since it increased the ketonuria of diabetic rats fed a carbohydrate-free high-protein diet (3).

CONCLUSIONS

Adrenocorticotropic hormone increases both the urinary glucose and nitrogen excretion of hypophysectomized-diabetic rats. Growth hormone is without effect upon the urinary glucose but reduces the urinary nitrogen excretion of hypophysectomized-diabetic rats.

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EFFECT OF PARENTERAL ADMINISTRATION OF GLUCOSE AND PROTEIN HYDROLYSATE ON FOOD INTAKE IN THE RAT

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ONE of the most remarkable adjustments which animals and men make is the regulation of food intake in accordance with bodily needs. The fact that most animals show a high constancy in body weight, even when large variations in caloric expenditure occur, indicates that food intake is normally closely attuned to caloric need.

An analysis of the factors concerned in this regulation is the study of hunger and appetite.

The simplest form that this regulatory mechanism could take would be for each unit expenditure of energy to reflect itself in a unit drive to eat. The resultant intake of a unit quantity of food of unit potential energy would then balance the existing caloric deficit and restore caloric equilibrium. Variation of expenditure of energy, and potential energy of available food stuffs, would be directly reflected in variation of intake of food.

If such a regulatory mechanism were sensitive to relatively small caloric deficits, it would tend to restore these deficits from meal to meal, or from day to day, depending on the eating habits of the species involved.

In this theoretical manner, caloric expenditure and food intake would be nicely balanced at a rapid rate with constancy of body weight.

It would also follow that supplying the animal economy with units of potentially available energy in the form of parenteral administration of calorically valuable material would result in the rapid restoration of caloric equilibrium by the diminution of food intake by comparable units.

The experiments reported in this paper were designed to test this hypothesis by noting the effects of intraperitoneal injection of glucose and protein hydrolysate mixtures upon the food intake of the rat.

METHODS

Ten male white rats grown in this laboratory ranging in weight from 180 to 300 gm. were housed in individual cages in a relatively constant temperature room, with free access to water at all times.

The animals were divided into two groups of 5 rats each, according to their basal diet. The first group, series A, was maintained on a fluid diet containing 5 per cent glucose and 5 per cent protein hydrolysate (amigen). The fluid diet was given by the conventional drinking bottle. It supplied 0.35 cal/cc. A daily vitamin supplement in liquid form consisting of thiamine hydrochloride 20 μg, riboflavin 25 μg, pyridoxine 20 μg, calcium pantothenate 100 μg and nicotinic acid 100 μg was fed by mouth.

The second group of animals, series B, was maintained on an adequate commercial dry food (Purina, Chow) and supplemented by the vitamin mixture as above. The chow was supplied in a small glass jar placed within a deeper jar and thereby scattering was held to a minimum. This diet supplied 3.3 cal/gm.

The 24-hour intake of food was measured daily at the same hour. The animals were weighed daily, and all gained weight during the period of observation.

The solutions used for intraperitoneal injection were 1) 0.5 per cent NaCl as a control, 2) 10 per cent glucose in 0.3 per cent NaCl and 3) 5 per cent glucose-5 per cent amigen mixture. On treatment days one of these solutions was injected two times during the day, 15 cc. each time, except in one animal where 20 cc. were injected each time. The order of administration of the solutions was randomized and a day of rest intervened between each day of treatment. Each test solution was used in each rat on three to seven test days.

RESULTS

Series A. Rats on Liquid Diet

From the results summarized in table 1 it is readily apparent that some depression of food intake occurred in all treatment periods, including those in which saline alone was administered. The average reduction in food intake associated with saline injection was as large as that observed with the injection of foodstuffs. The range of decrement of caloric intake attending the injection of 30 cc. of 5 per cent glucose-5 per cent amigen (from 3.5 cal/day to 10.85 cal/day) and of 30 cc. of 10 per cent glucose (from 1.05 cal/day to 10.2 cal/day) did not exceed that produced by 30 cc. of 0.5 per cent saline (from 3.15 cal/day to 16.7 cal/day). In only 2 rats did the average depression of food intake by both glucose and glucose-amigen mixtures exceed that produced by saline. Further, only in *rat A₃* did the average depression of calories ingested equal or exceed the caloric value of the injected material.

Series B. Rats on Stock Diet

The results summarized in table 2 indicate that although some depression of average food intake occurred in most instances, this was not a constant finding. In one instance no depression took place and in 2 animals a slight increase in food intake was noted. In 4 animals depressions of average food intake were induced by saline as well as by the nutrient materials. The range of change induced by 30 cc. of 5 per cent amigen-5 per cent glucose (from 0 to −8.6 cal/day) and by 30 cc. of 10 per cent glucose in 0.3 per cent saline (from +0.3 cal/day to −7.3 cal/day) did not exceed the range of changes induced by 30 cc. of 0.5 per cent NaCl (from +3 cal/day to −11.2 cal/day).

In no animal did the depression of food intake by both glucose and glucose + amigen mixtures exceed that produced by saline. Further, only in *rat B₂* did the depression of calories ingested equal or exceed the caloric value of the material injected. None of the variations in the degree of depression, produced by the various intraperitoneal injections, are of sufficient magnitude to be significant.

DISCUSSIONS

These experiments indicate that the depression of food ingestion induced in rats by the intraperitoneal injection of 10 per cent glucose and 5 per cent glucose + 5 per cent amigen mixtures is of the same order of magnitude as that induced by injection

of comparable volumes of saline, and that this decrement is not related to the caloric value of the materials injected. Within the periods of this experiment administration of from 17 to 25 per cent of the daily caloric requirement of the rat did not diminish food intake beyond that produced by an equal volume of non-caloric material.

These findings are in keeping with the failure of intravenous glucose to inhibit

TABLE 1

ANIMAL NO.	PERIOD	VOL. GIVEN I.P.	NO. OF DAYS	CAL. GIVEN I.P.	VOL. OF DIET IN-GESTED DAILY	AVERAGE CAL. IN-GESTED DAILY	CHANGE FROM CONTROL PERIOD
		cc.			cc.		*Cal/day*
A_1	Control		24		175	61.35	
	NaCl[1]	30	5	0	154	53.9	−7.4
	Amigen[2]	30	5	10.5	152	53.2	−8.1
	10% glucose[3]	30	5	12	146	51.1	−10.2
A_2	Control		18		171	59.85	
	NaCl	30	4	0	125	43.75	−16.7
	Amigen	30	4	10.5	161	56.35	−3.5
	10% glucose	30	5	12	145	50.75	−9.1
A_3	Control		17		173	60.55	
	NaCl	30	6	0	156	54.60	−5.95
	Amigen	30	6	10.5	142	49.70	−10.85
	10% glucose	30	6	12	166	58.1	2.45
A_4	Control		15		168	58.80	
	NaCl	30	4	0	157	54.95	−3.85
	Amigen	30	5	10.5	147	51.45	−7.35
	10% glucose	30	5	12	150	52.5	−6.30
A_5	Control		21		173	60.55	
	NaCl	30	4	0	164	57.40	−3.15
	Amigen	30	3	10.5	154	53.90	−6.60
	10% glucose	30	3	12	170	59.50	−1.05

[1] 0.5% NaCl.   [2] 5% glucose-5% protein hydrolysate (amigen).   [3] 10% glucose in 0.3% NaCl.

food intake in the dog (1), and with the previously reported failure of intravenously administered fat emulsions in the dog (2), and certain amino acid mixtures in man (3) to inhibit food intake in short-term experiments. This interpretation is at variance with that recently reported by Adolph (4). This observer noted that injected nutrients reduced the voluntary intake of food by mouth in the rat and concluded that "Plethora inhibits eating . . ., even when the alimentary tract has not been concerned in the creation of the plethora". However it should be noted that the effects of injection of non-caloric material were not studied and detailed protocols were not furnished.

The results detailed in this study do not support the hypothesis of a simple regulatory mechanism outlined in the introduction above, in which variation of caloric requirement would be easily and rapidly balanced by variation in food intake, from meal to meal, or from day to day.

Caloric requirements, decreased by the parenteral administration of calorically

TABLE 2

ANIMAL NO.	PERIOD	VOL. GIVEN I.P.	NO. OF DAYS	CAL. GIVEN I.P.	AVERAGE CAL. EATEN DAILY	CHANGE FROM CONTROL PERIOD
		cc.				*Cal/day*
B_1	Control		28		61.7	
	NaCl[1]	30	7	0	53.4	−8.3
	Amigen[2]	30	7	10.5	53.1	−8.6
	10% glucose[3]	30	7	12	54.8	−6.9
B_2	Control		21		78.3	
	NaCl	40	4	0	49.5	−28.7
	Amigen	40	4	14	53.4	−24.8
	10% glucose	40	4	16	51.8	−26.4
B_3	Control		27		45.5	
	NaCl	30	5	0	34.3	−11.2
	Amigen	30	5	10.5	40.2	−5.3
	10% glucose	30	5	12	38.2	−7.3
B_4	Control		25		49.8	
	NaCl	30	6	0	52.8	+3
	Amigen	30	7	10.5	49.8	0
	10% glucose	30	7	12	49.2	−1.6
B_5	Control		22		51.2	
	NaCl	30	6	0	43.3	−7.9
	Amigen	30	6	10.5	47.2	−4.0
	10% glucose	30	6	12	51.5	+0.3

[1] 0.5% NaCl. [2] 5% glucose-5% protein hydrolysate (amigen). [3] 10% glucose in 0.3% NaCl.

valuable material, were not balanced by corresponding decrement of food intake as measured from day to day.

The regulatory mechanism would seem either not to be sensitive to variations of caloric requirement within this range (up to 25%), or else to respond to these variations at a much slower rate than that presupposed by any day to day, or meal to meal readjustment. In view of the precision which normally occurs in adjustment of food intake to energy expenditure, and the high constancy of body weight, it appears most likely that such adjustments are not made from meal to meal, or from day to day, but require longer periods of time than those available to the animal in this set of experiments.

SUMMARY AND CONCLUSIONS

The intraperitoneal administration of glucose and protein hydrolysate mixtures to the rat in calorically significant amounts did not produce an equivalent depression of food intake. The depression which did occur was not greater than that produced by saline injections. The normal regulatory mechanisms responsible for the precise adjustment of food intake to caloric requirement are not operative during the short period of these experiments.

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A DIETARY FACTOR REGULATING THE ENZYME CONTENT OF THE PANCREAS: CHANGES INDUCED IN SIZE AND PROTEOLYTIC ACTIVITY OF THE CHICK PANCREAS BY THE INGESTION OF RAW SOY-BEAN MEAL[1]

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IT IS shown in the present communication that the feeding of raw soy-bean meal induces profound changes in the chick pancreas not only in its size but also in its trypsin content. This finding is of particular interest, for the failure of raw soy-bean meal to support maximal growth has been ascribed to a trypsin inhibitor (1), which has been isolated from the bean in crystalline form (2). Concentrates of this inhibitor retard growth even when added to diets containing nutritionally adequate animal proteins (1, 3). On the basis of the findings presented here it is postulated that the acinar tissue of the pancreas is stimulated to increased activity by the presence of dietary proteolytic inhibitors in the intestinal tract.

EXPERIMENTAL

Treatment of Animals

The White Leghorn chicks used in each experiment were obtained from a single hatch. They were kept in electrically heated battery-brooders. For the first week after hatching they were fed a 'starter' ration[2]; thereafter groups of these chicks whose weights were in close agreement were fed soy-bean diets. Raw or heated soy-bean meal served as the source of protein in these diets. The beans were ground in a corn mill at room temperature. The *cooked* soy beans were prepared by heating the ground beans in an autoclave at one-pound pressure for 45 minutes. The raw and cooked soy-bean diets used in each experiment were prepared from a single batch of beans. Since the batches of soy beans varied somewhat in their protein contents, the desired protein composition of each diet was obtained by varying the proportions of soy-bean meal and cerelose. A typical example of the composition of the diets used in this investigation is recorded in table 1.

Chicks were sacrificed by dislocation of the cervical vertebra. Their entire

[2] The starter ration had the following composition: corn, 300 parts; wheat, 135; barley, 100; alfalfa meal, 40; wheat bran, 150; sardine meal, 75; soy-bean meal, 100; dried whey, 25; dried skim milk, 25; liver meal, 20; oyster shell, 15; bone meal, 12; salt, 5; manganese sulfate, 0.5; Sardilene (a fish oil containing 400 units of Vitamin D and 3000 units of Vitamin A per cc.), 2.5 parts. To each 1000 lbs. of the above mixture, one gram of riboflavin was added.

pancreas were rapidly removed, freed of extraneous tissue, immediately chilled, and weighed. Various other organs were also excised and weighed.

The chilled pancreases were transferred to a tube (kept cold by immersion in an ice-salt bath) and homogenized with a few cc. of ice-cold distilled water. The homogenate was then diluted with distilled water to yield a concentration of about 20 mg. of tissue per cc. Unless analyses of the homogenate were carried out immediately, it was stored at $-15°C$. No conversion of trypsinogen to trypsin occurred under these conditions, since proteolytic activity of homogenates was found to be negligible in the absence of added enterokinase.

Determination of Proteolytic Activity of Pancreas Homogenates

As stated above, pancreas homogenates do not digest proteins in the absence of enterokinase. For example, when as much as 5 mg. of chick pancreas was added to denatured hemoglobin substrate (see below), no detectable proteolysis occurred. After incubation with enterokinase, however, as little as 0.05 mg. of pancreas digested measurable quantities of hemoglobin. The increase in the proteolytic power

TABLE I. COMPOSITION OF TYPICAL DIETS (EXPERIMENT 3)

DIETS	SOY BEAN MEAL	CERELOSE	YEAST	VITAMIN-SALT SUPPLEMENT[1]
	%	%	%	
10% protein..........................	24.9	65.85	5	4.25
25% protein..........................	62.3	28.45	5	4.25

[1] This supplement had the following composition: Sardilene (a fish oil containing 400 units of vitamin D and 3000 units of vitamin A per cc.) 6%, $CaCO^3$ 24%, $Ca^3(PO_4)_2$ 47%, salt mixture 23%, riboflavin 0.2 mg. per 100 gm. of mixture.

of the homogenate is due to conversion of the proenzymes (trypsinogen and chymotrypsinogen) to active proteinases (trypsin and chymotrypsin). The conditions which favor this conversion have been extensively studied by Kunitz (4). On the basis of his findings preliminary experiments were performed to determine the optimal conditions for activation of the proteolytic activity of pancreas homogenates. Such factors as temperature, pH, concentration of enterokinase, concentration of pancreas, and period of incubation were studied. The following method was then developed for determination of proteolytic activity of the pancreas homogenates.

Activation of Pancreatic Proteinases by Enterokinase. Purified enterokinase was prepared from the duodenal mucosa of dogs by the method of Kunitz (4). The material derived from one gm. of mucosa was dissolved in one cc. of a 0.02M phosphate buffer (pH 7.6). This solution was stored at $-15°$ and was used throughout the experiments reported here.

Before use the temperature of the enterokinase solution was raised to $0°$ and an aliquot of it diluted 1:50 with cold 0.02M phosphate buffer (pH 5.8). The pancreas homogenates were also diluted with this same buffer so that each cc. contained about 2 mg. of pancreas. Five cc. of the diluted enterokinase solution, 2 to 5 cc. of the diluted pancreas suspension and sufficient 0.02M phosphate buffer (pH 5.8) to make

a total volume of 15 cc. were transferred to each of several glass-stoppered Erlen-meyer flasks. The flasks were then placed in a constant temperature bath at 37.5°C. and shaken for 15 minutes. Periods of activation longer than 15 to 30 minutes were found to result in a gradual decline of the proteolytic activity of the mixture.

Immediately following its activation, the pancreas suspension was made up to a volume of either 25 or 50 cc. by the addition of cold 0.02M phosphate buffer (pH 7.6) and rapidly chilled to 0°. The suspension was kept at 0° until samples were taken for determination of its proteolytic activity (less than 1 hr. later). In some cases several aliquots were diluted with the pH 7.6 buffer to provide a range of pro-teolytic activities. As a rule, the samples of activated solution used for the deter-mination of proteolytic activity contained 0.1 to 0.5 mg. of pancreas, the amount varying, of course, with the concentration of proteinases.

Measurement of Proteolysis. Anson's method (5) was employed for the de-termination of the proteolytic activity of the activated pancreas solutions. It in-volves the hydrolysis of denatured hemoglobin (prepared from beef corpuscles) and the measurement of its split products (largely peptides) which are soluble in tri-chloracetic acid. The substrate solution contained about 2 per cent of the denatured hemoglobin in phosphate buffer (pH 7.6). One-cc. aliquots of the activated pancreas solution and 5 cc. of hemoglobin solution were incubated for 10 minutes at 37.5°. The reaction was terminated by the addition of 10 cc. of 0.3 N trichloracetic acid. The filtrate obtained was alkalinized and treated with the phenol reagent (5), the color was allowed to develop for 5 to 10 minutes and measured by a photoelectric colorimeter. A red filter (Klett-Summerson No. 66) was used. The color values for the substrate-pancreas mixtures at zero time were determined by adding enzyme solution to a mixture of the substrate and trichloracetic acid.

Control determinations were performed to ascertain the color products resulting from autolysis of pancreas, digestion of hemoglobin by enterokinase alone, and non-enzymatic breakdown of hemoglobin. These corrections were very small and conse-quently were ignored without introducing an error greater than 5 per cent.

The unit of proteolytic activity is defined by Anson as "The amount which digests hemoglobin under the standard conditions at an initial rate such that there is liberated per minute an amount of split products not precipitated by trichloracetic acid which gives the same color with the phenol reagent as one milliequivalent of tyrosine". The color values of standard tyrosine solutions were determined and compared with the values obtained by proteolysis. The trypsin units per gm. pancreas were then calculated. Trypsin units as used in this study imply measure-ment of proteolytic activity at pH 7.6. No attempt was made to distinguish be-tween the proteolytic activities of trypsin and chymotrypsin.

The nitrogen content of the pancreases was determined by the semi-micro Kjel-dahl method.

<center>RESULTS</center>

<center>*Body and Organ Weights*</center>

Body and Pancreas Weights of Chicks Fed Raw and Cooked Soy-Bean Protein Diets. In *experiment 1* all birds were killed at three weeks of age, and during the

last two weeks they were fed diets containing 10 to 25 per cent raw or autoclaved soy-bean protein (table 2).   A comparison of the weights of birds fed the four levels of soy-bean protein brings out again the effect of heating on the biological value of this protein.   This is particularly evident at the higher levels of intake.   Thus, in *experiment 1*, the birds fed a diet containing 20 per cent raw soy-bean protein weighed

TABLE 2.   INFLUENCE OF SOY BEAN DIETS ON THE SIZE AND ENZYME CONTENT OF THE PANCREAS

						PANCREAS[2]			
EXP.	NO. OF CHICKS USED	PERIOD ON DIET[1]	CHICK WT.[2]	PROTEIN CONTENT OF DIET	CONDITION OF SOY BEAN MEAL	Total wet wt.	Per cent of body wt.	Proteolytic activity[3]	
								Per gm. fresh pancreas	Per gm. pancreas nitrogen
		days	*gm.*	%		*gm.*		*units*[4]	*units*[4]
	4	14	70	10	Raw	0.47	0.63	0.07	3.1
	4	14	80	10	Cooked[5]	0.33	0.42	0.07	2.5
	3	14	65	15	Raw	0.33	0.55	0.23	8.6
1[6]	4	14	145	15	Cooked	0.61	0.42	0.15	5.5
	4	14	81	20	Raw	0.54	0.65	0.20	7.0
	4	14	149	20	Cooked	0.65	0.45	0.13	5.0
	4	14	106	25	Raw	1.02	0.95	0.29	10.3
	4	14	167	25	Cooked	0.75	0.44	0.27	8.6
	3	23	91	10	Raw	0.66	0.74	0.25	10.1
	5	23	115	10	Cooked	0.55	0.48	0.21	7.4
2[6]	5	23	116	18	Raw	0.80	0.66	0.26	9.3
	5	23	176	18	Cooked	0.77	0.44	0.19	6.7
	5	23	158	26	Raw	1.41	0.88	0.46	14.5
	5	23	265	26	Cooked	1.11	0.42	0.23	7.9
	10	23	56	10	Raw	0.46	0.82	0.11	4.8
3[6]	10	23	92	10	Cooked	0.51	0.56	0.07	2.7
	10	23	116	25	Raw	1.21	1.04	0.38	11.7
	10	23	190	25	Cooked	0.89	0.47	0.18	6.1

[1] For the first week after hatching the chicks were fed a 'starter' ration; thereafter, they received the experimental diet.   The chicks in *exp. 1* were 21 days old when killed; those in *exp. 2* and *3* were 30 days old when killed.

[2] Each value is the average of 3–10 closely agreeing results obtained from as many birds.

[3] A measure of both trypsin and chymotrypsin activity (see text).

[4] Trypsin units as defined by Anson (5).   See text.

[5] Autoclaved at 1 lb. steam pressure for 45 min.

[6] These are the same experiments as those recorded in table 4.

81 gm. (average), whereas those that received the diet containing the same percentage of autoclaved protein weighed 149 gm. (average).   The weight difference was equally striking in birds fed diets containing 26 per cent protein (*experiment 2*).

In chicks fed the autoclaved soy-bean diet, the amount of pancreas tissue per 100 gm. body weight remained quite constant.   Thus in birds whose average weights varied from 80 to 167 gm. (*experiment 1*) the average pancreas values were 0.42,

0.42, 0.45, and 0.44 for the four groups that received respectively 10, 15, 20, and 25 per cent protein diets.   The birds fed the same levels of protein derived from *raw* soy-beans weighed 65 to 106 gm. (averages) at three weeks of age; the average weights of their pancreases were respectively 0.63, 0.55, 0.65, and 0.95 gm. per 100 gm. body weight.

In *experiments 2* and *3* the birds were sacrificed at 30 days of age.   The results obtained in these two experiments (table 2) show again that birds fed diets containing *raw* soy-beans as the sole source of protein have larger pancreases than those fed the autoclaved soy-diets.

In table 3 are recorded pancreas weights for birds fed a normal diet, namely the U.C. stock ration[3].   The average values did not exceed 0.56 per cent of their body weights.   Latimer (6) also found that pancreases in chicks weighing between 50 and 250 gm. and fed a normal mixed diet amounted to about 0.5 per cent of their body

TABLE 3.   ORGAN WEIGHTS AND PROTEOLYTIC ACTIVITY OF THE PANCREAS OF CHICKS FED THE U.C. STOCK RATION[1] (EACH VALUE IS THE AVERAGE OF 5–8 CLOSELY AGREEING RESULTS OBTAINED FROM AS MANY BIRDS)

NO. OF CHICKS USED	CHICK WT.	CHICK AGE	ORGAN WT. AS PERCENTAGE OF BODY WT.			TRYPTIC ACTIV- ITY/GM. PANCREAS NITROGEN
			Pancreas	Liver	Heart	
	gm.	*days*				*units[2]*
8	166	28	0.52			8.9
8	202	30	0.56	3.2	0.68	8.4
8	223	30	0.51			
5	220	30	0.47	2.7		7.5

[1] The diet had the following composition: corn, 300 parts; wheat, 135; barley, 100; alfalfa meal, 40; wheat bran, 150; sardine meal, 75; soy bean meal, 100; dried whey, 25; dried skim milk, 25; liver meal, 20; oyster shell, 15; bone meal, 12; salt, 5; manganese sulfate, 0.5; fish oil, 2.5.   Ribo-flavin was added to the extent of 1 gm/1000 lbs. of diet.

[2] Trypsin units determined by the method of Anson (5).

weights.   In view of these observations, it seems reasonable to conclude that hyper-trophy of the pancreas had occurred in the birds fed the *raw* soy-bean meal.

Liver and Heart Weights of Birds Fed Raw and Autoclaved Soy-Bean Diets. That the increase in size of the pancreas in chicks fed a raw soy-bean diet is spe-cific for only this organ is brought out in figures 1, 2, and 3.   In these, organ weights were plotted against body weights.   The data were obtained from *experi-ments 2* and *3*.   In *experiment 3*, for example, the weights of the livers and hearts of chicks fed raw soy did not vary consistently from those fed autocalved soy.   Nor in *experiment 2* was there a consistent difference in the liver weights between the chicks fed raw and those fed autoclaved soy.

Effect of Supplemental Methionine on Pancreas Size and Body Weight of Chicks. Since methionine corrects to a large extent the deficiencies induced by a *raw* soy-bean diet (7), the effect of supplementing the raw soy-bean diets with this amino acid upon pancreas size was investigated.   The results are recorded in table 4.

[3] Its composition is described in table 3.

.The addition of 0.5 per cent methionine to the *raw* soy-bean diets, even when the protein contents of the diets were as high as 25 to 26 per cent, resulted in marked stimulation in the growth of the chicks. Thus 30-day old chicks fed the raw soy-bean diet containing 26 per cent protein for three weeks had an average weight of 158 gm. (*experiment 2*); those fed the same diet plus 0.5 per cent methionine weighed 267 gm. (average).

TABLE 4.   EFFECT OF SUPPLEMENTAL METHIONINE ON THE SIZE AND ENZYME CONTENT OF THE PANCREAS OF CHICKS FED SOY BEAN DIETS

SOY BEAN MEAL	EXP.[6]	NO. OF CHICKS USED	PERIOD ON DIET[1]	CHICK WT.[2]	PROTEIN CONTENT OF DIET	METHIO-NINE ADDED	PANCREAS[2]			
							Total wet wt.	Per cent of body wt.	Proteolytic activity[3]	
									Per gm. fresh pancreas	Per gm. pancreas nitrogen
			days	*gm.*	*%*	*%*	*gm.*	*gm.*	*units*[4]	*units*[4]
Raw	1	4	14	106	25	None	1.02	0.95	0.29	10.3
		4	14	146	25	0.5	1.49	1.03	0.37	10.9
	2	5	23	158	26	None	1.41	0.88	0.46	14.5
		5	23	267	26	0.5	2.33	0.87	0.34	10.4
	3	10	23	116	25	None	1.21	1.04	0.38	11.7
		10	23	163	25	0.5	1.77	1.05	0.46	13.1
Cooked[5]	1	4	14	167	25	None	0.75	0.44	0.27	8.6
		4	14	175	25	0.5	0.70	0.40	0.15	4.8
	2	5	23	265	26	None	1.11	0.42	0.23	7.9
		5	23	258	26	0.5	1.02	0.39	0.18	5.8
	3	10	23	190	25	None	0.89	0.47	0.18	6.1
		10	23	206	25	0.5	0.95	0.46	0.22	7.1

[1] For the first week after hatching chicks were fed a 'starter' ration, thereafter they received the experimental diets recorded in table 1.
[2] Each value is the average of −10 closely agreeing results obtained from as many birds.
[3] A measure of both trypsin and chymotrypsin activity (see text).
[4] Trypsin units as defined by Anson (5).
[5] Autoclaved at 1 lb. steam pressure for 45 min.
[6] These are the same experiments as those recorded in table 2.

The increase in body weight produced by supplementing the raw soy-bean diet with 0.5 per cent methionine was accompanied by an increase in pancreas size, but the ratio of pancreas weight to body weight was *not* changed; in both groups of birds the pancreas weights amounted to 0.9 to 1.0 per cent of their body weights.

The addition of methionine to diets containing 25 or 26 per cent autoclaved soy-bean protein as the source of protein failed to increase the body weights of the chicks. The pancreas weights of the birds fed these same levels of *autoclaved* soy

protein with or without supplemental methionine did not exceed 0.5 per cent of their body weights.

Proteolytic Activity of Pancreas

Proteolytic Activity of Pancreases of Chicks Fed Raw and Autoclaved Soy Beans. Values for proteolytic activity of the pancreas as determined by the procedure of
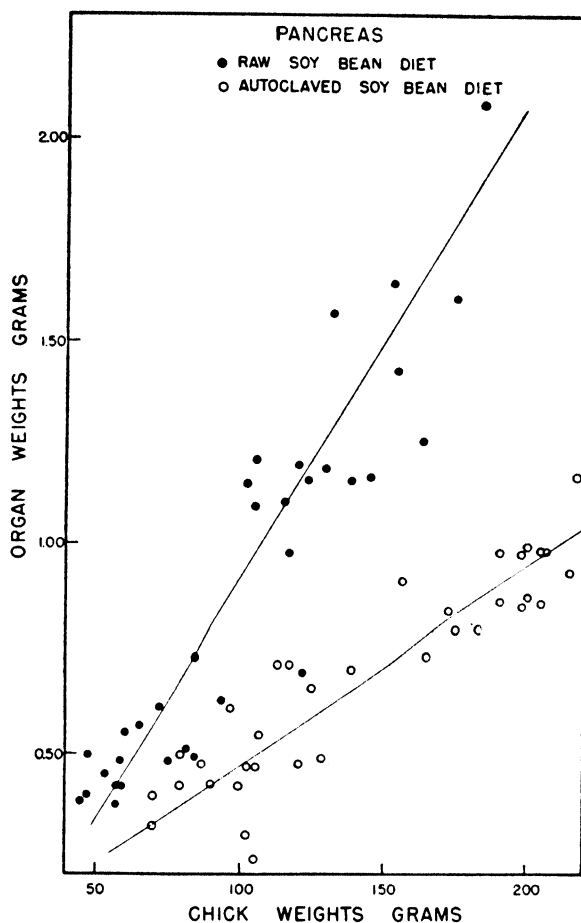
Fig. 1. PANCREAS WEIGHTS OF CHICKS fed raw and autoclaved soy-bean diets. The data were obtained from *experiments 2* and *3*.

Anson are recorded in table 2. As already pointed out, this method measures combined tryptic and chymotryptic proteolysis. The tryptic content expressed as either per gm. of fresh pancreas or per gm. of nitrogen was greater in birds fed raw than in those fed autoclaved soy-bean meal. Thus the pancreases of the chicks of *experiment 3*, which were fed a raw soy-bean diet providing 25 per cent protein, contained 11.7 trypsin units per gm. of nitrogen (average value), whereas those fed a diet with the same level of autoclaved soy-bean protein contained an average of 6.1 units per gm.

nitrogen.   The differences in the proteolytic contents of the pancreases between birds fed raw and those fed autoclaved soy-bean diets were equally striking when the results were compared in terms of the whole gland or per kilo of body weight.

Effect of Supplementing Soy-Bean Diets with Methionine upon Proteolytic Activity of Chick Pancreas.   Although supplemental methionine increased the weights of chicks fed raw soy beans at the 25 per cent protein level, table 4 shows that it did not change the concentration of proteolytic enzymes in the pancreas.
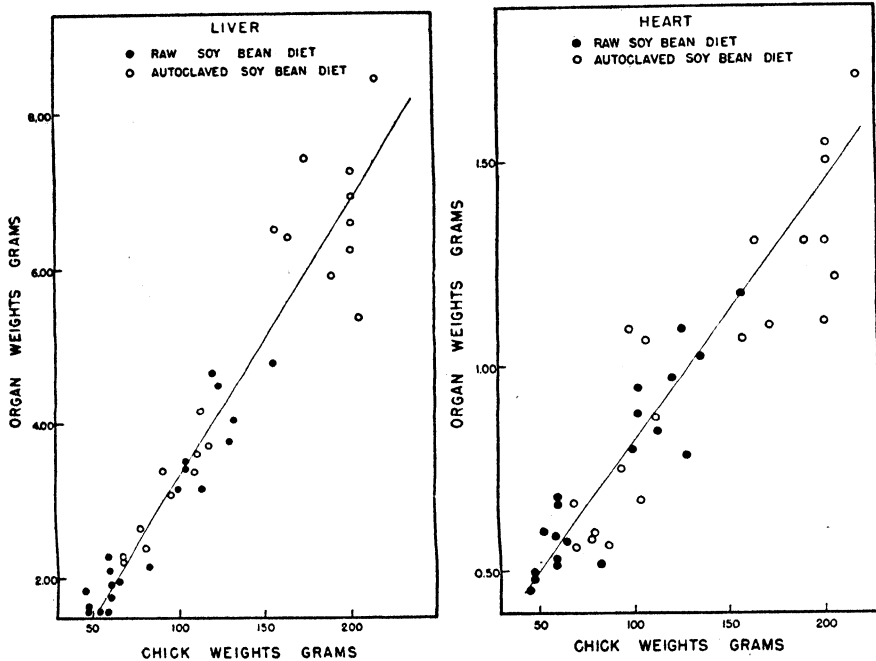
Fig. 2 *(left)*.   LIVER WEIGHTS OF CHICKS fed raw and autoclaved soy-bean diets.   The data were obtained from *experiments 2 and 3.*

Fig. 3 *(right)*.   HEART WEIGHTS OF CHICKS fed raw and autoclaved soy-bean diets.   The data were obtained from *experiment 3.*

The addition of 0.5 per cent methionine to the cooked soy-bean diet influenced neither the size nor the proteolytic activity of the chick pancreas.

<div align="center">DISCUSSION</div>

The amount of pancreas in chicks fed a normal diet or a diet containing auto-claved soy beans seldom exceeded 0.5 per cent of their body weights.   The values found in birds fed raw soy-bean diets, however, were frequently greater than one per cent.   The pancreas responded to the raw soy bean not only in size but in enzyme concentration as well.   The concentrations of tryptic activity per gm. of fresh pancreas or per gm. of pancreas nitrogen were greater in chicks fed the raw meal than in those fed the autoclaved meal.   This is particularly evident at the higher levels of protein intake, namely 25 to 26 per cent.   Supplemental methionine, which at a

high level of protein intake improved the weights of chicks fed the *raw* soy-bean meal, failed to prevent the pancreas hypertrophy or the increased concentration of proteolytic enzymes in this gland.

The presence in raw soy-beans of a proteolytic-inhibiting substance was first demonstrated by Bowman (8) and subsequently confirmed by Ham *et al.* (9). Recently Kunitz (2) has isolated from this bean a heat-labile crystalline protein which can combine with an equal weight of crystalline trypsin to form a stable compound having no proteolytic activity. The trypsin-inhibiting action of the soy protein is decreased by proper heat-treatment. It is therefore proposed as a working hypothesis that the increase in size and tryptic content of the pancreas observed here in the birds fed the raw soy bean results from stimulation of the acinar tissue of this gland by either the inhibitor *per se* or by a product of incompletely digested protein. The recent affirmation by Thomas (10) and Ramsay (11) that the presence of peptones in the intestinal tract increases the secretion of the acinar tissue of the pancreas lends support to this hypothesis.

SUMMARY

The prolonged feeding of raw soy-bean meal induced in chicks an enlargement of the pancreas and an increase in its proteolytic content. Methionine corrected the growth defect observed in the bird fed a raw soy-bean diet, but failed to prevent the hypertrophy of the pancreas or the increase in its activity. A possible explanation of the pancreas hypertrophy is offered.

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EFFECT OF PARATHYROID ON RENAL TUBULAR REABSORPTION OF PHOSPHATE AND CALCIUM[1]

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THE mechanism of action of parathyroid hormone has been the subject of considerable controversy. One of the two main schools of thought holds that it primarily affects the metabolism of phosphorus, the other, that it primarily affects the metabolism of calcium.

Albright (1) has proposed the theory that the hormone, by increasing the urinary loss of phosphate, leads to hypophosphatemia. Because of the reduction in phosphate level, the serum becomes unsaturated with respect to calcium phosphate and, as a consequence, calcium enters the serum from the gastro-intestinal tract and from the bones in increased quantities. Hypercalcemia and hypercalcuria result. Ellsworth (2) maintains that the hyperphosphaturia which follows parathyroid hormone is caused by a lowering of the renal threshold for phosphorus. These investigators as well as others (3–7) have presented clinical as well as experimental evidence in favor of the view that all, or at least a major part, of the action of the hormone is exerted through its effect upon the renal reabsorption of phosphate.

In contrast Selye (8) believes that the hormone acts primarily by stimulating the formation and activity of the osteoclasts of bone, with the result that calcium and phosphorus are mobilized in increased quantities from skeletal stores. Increased mobilization and elevated serum levels account for the increased excretion of both ions. Later Collip *et al.* (9), in a study of the bones of nephrectomized rats that had been injected with parathormone, concluded that the action of the hormone on the bones is independent of any direct influence it may have on the renal threshold for phosphorus.

For a better understanding of the overall action of parathyroid hormone, an exact definition of its effect on the renal tubular reabsorption of phosphate is essential. So far the findings of the various groups that have studied the problem directly have been contradictory. Harrison and Harrison (10) studied reabsorption over a very narrow range of plasma phosphate concentration and concluded that the hormone depresses phosphate reabsorption. Marion Fay and her associates (11) who studied phosphate/creatinine clearance ratios over a wide range of plasma concentration in normal, parathyroid treated, and parathyroidectomized dogs inferred that a lack or an excess of the hormone produced no demonstrable effect upon the capacity of the kidney to excrete, and hence presumably to reabsorb, phosphate.

We felt that the problem merited a thorough investigation; particularly did we feel that the renal tubular reabsorption of inorganic phosphate, in normal and parathyroid treated animals should be studied over a wide range of plasma phosphate concentration and under rigidly controlled conditions of parathyroid activity. The experiments reported below were designed to study this problem.

METHODS

Twelve experiments were performed to assess the effect of parathyroid hormone on the renal tubular reabsorption of inorganic phosphate: 6 with and 6 without the hormone, the latter serving as controls. Eight additional experiments were performed to study the effect of the hormone on calcium reabsorption. All experiments were performed on 2 female mongrel dogs loosely restrained on a comfortable animal board. Preliminary observations were made to find a means of administering the hormone which would ensure a significant increase in serum calcium concentration, stable for the duration of the experiment, and yet exert no gross effect on renal function.[3] The following technique of Collip (12) was found to be most advantageous. Parathyroid extract (Lilly)[4] was given subcutaneously in 2 injections of 3 ml. (U.S.P. 300 U) separated by an interval of 6 hours, starting about 20 hours before the experiment. With this regimen a high and uniform serum-calcium level was obtained at the time the experiment was performed, a level which averaged 13 to 14 mg. per cent, the normal being 8 to 10 mg. per cent. Accordingly the hormone was injected at 12 noon and at 6 P.M. and on the following morning the experiment was performed.

The animals were maintained on a diet of dog biscuits supplemented with meat twice a week. Water, in amounts of 50 ml/kilo body weight, was administered by stomach tube about an hour before the experiment to ensure adequate hydration of the animal. Urines were collected by an indwelling catheter and the bladder was washed with distilled water at the end of each urine collection period. All periods were 10 minutes in length. Two samples of blood, one with a..d the other without oxalate, were drawn from the jugular vein at the mid-point of each urine collection period. Plasma was used for the analysis of creatinine and phosphate, and serum for calcium. A continuous infusion was administered by the saphenous vein at a rate of 5 ml/min. In the first 12 experiments the infusions contained sodium phosphate (pH 7.4) in quantities sufficient to raise the plasma level in stepwise fashion from a normal value to 1 mM to 5 mM/l., creatinine for the measurement of glomerular filtration rate, and distilled water or 0.9 per cent sodium chloride to render the solution roughly isotonic. In the second group of experiments phosphate was omitted from the infusions.

Creatinine was determined on iron filtrates of plasma and diluted urine by the method of Folin and Wu (13). Phosphate was determined on trichloracetic acid filtrates of plasma and diluted urine by the method of Fiske and Subbarow (14) as modified by Pitts (15). Calcium was determined on trichloracetic acid filtrates of serum and evaporated urine by the method of Kramer and Tisdall (16) as modified by Stanford (17), after precipitating the calcium oxalate overnight. All colorimetric analyses were carried out with an Evelyn photoelectric colorimeter.

RESULTS

The amount of phosphate reabsorbed by the renal tubules in one minute has been calculated as the difference between the quantity filtered through the glomeruli

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[3] Large doses of parathormone administered repeatedly reduce glomerular filtration rate and may cause the formation of multiple infarcts in the kidneys.

[4] We are indebted to Eli Lilly and Co. for a generous supply of the hormone.

and the quantity excreted in the urine.   The quantity filtered was determined as the product of the glomerular filtration rate in ml/min. and the plasma concentration of phosphate in mM/ml.   The quantity excreted was determined as the product of the urine flow in ml/min. and the urinary concentration of phosphate in mM/ml.

The data obtained in 2 typical experiments on one dog, the first a control, and the second following parathormone, are illustrated in table 1.   The first 2 horizontal rows of figures in each experiment are data obtained prior to the infusion of phosphate, the remaining 6 rows are data obtained during the infusion of phosphate.

TABLE 1.   EXPERIMENTS ILLUSTRATING RELATIONSHIP BETWEEN QUANTITIES OF INORGANIC PHOS-PHATE FILTERED THROUGH THE GLOMERULI AND QUANTITIES REABSORBED BY TUBULES AND EXCRETED IN THE URINE IN A NORMAL DOG AND IN THE SAME ANIMAL FOLLOWING ADMINISTRATION OF PARATHORMONE. *Dog P.*

URINE FLOW	GLOM. FILT. RATE	PHOSPHATE				CALCIUM	
		Plasma	Filtered	Excreted	Reabsorbed	Serum	Excreted
cc/min.	*cc/min.*	*mM/l.*	*mM/min.*	*mM/min.*	*mM/min.*	*mg.%*	*mg/min.*
			Control				
2.7	52.8	1.05	0.055	0.002	0.053	9.6	0.007
3.45	50.9	1.01	0.051	0.002	0.049	9.6	0.007
3.1	58.6	1.31	0.077	0.011	0.066	9.6	0.052
4.3	59.9	1.51	0.091	0.020	0.071	9.3	0.071
5.7	63.4	2.36	0.150	0.070	0.080	8.7	0.149
5.7	63.9	2.74	0.175	0.093	0.082	8.7	0.161
4.4	62.4	4.55	0.284	0.194	0.090	8.7	0.180
4.25	64.1	5.25	0.336	0.245	0.091	8.0	0.197
			With parathormone				
7.56	69.7	0.93	0.065	0.009	0.056	14.1	0.295
8.5	71.5	0.93	0.067	0.008	0.059	14.1	0.348
7.35	73.5	1.40	0.103	0.026	0.077	13.9	0.478
7.35	73.5	1.58	0.116	0.038	0.078	13.6	0.529
4.75	78.2	2.61	0.204	0.096	0.108	13.1	0.551
4.65	76.3	2.80	0.214	0.119	0.095	12.3	0.535
6.8	74.3	4.83	0.357	0.262	0.095	11.9	0.517
6.25	77.7	5.48	0.426	0.323	0.103	11.7	0.531

Plasma phosphate concentration was elevated in stepwise fashion from a normal level of about 1 mM/l. to 5 mM/l. by the infusion of solutions of increasing phosphate content.   Although glomerular filtration rate increased progressively throughout the experiment, both this variable and plasma level remained fairly constant in any pair of periods during which a given infusion was administered.

It is evident in the first 2 periods of the 2 experiments, prior to the administration of phosphate, that there was no significant difference either in plasma level or in rate of excretion of phosphate in the normal and in the parathormone treated state.   Thus we observe in the control experiment that the plasma phosphate level averaged 1.03 mM/l.; following parathormone it averaged 0.93 mM/l., a value essentially the same as the control.   Similarly the rate of excretion of phosphate amounted to 0.002 mM/

min. in the control experiment, a negligibly small value, and remained essentially unchanged at 0.009 mM/min. following parathormone.   In sharp contrast, the serum calcium level and the rate of excretion of calcium were considerably elevated by parathormone treatment.   Thus in the control experiment the serum calcium level was 9.6 mg. per cent; following the administration of hormone it was elevated to 14.1 mg. per cent.   The control rate of calcium excretion was 0.007 mg/min.; following the administration of hormone it rose to 0.348 mg/min.   Of most significance, however, is the fact that the normal and the hormone treated animal reabsorbed phosphate at rates which were identical within limits of experimental error over a fairly wide range of plasma phosphate concentration.   The maximum reabsorptive capacity (phosphate Tm) varied from 0.080 to 0.108 mM/min., a range no greater than that observed under normal conditions.   Indeed the higher values were obtained following hormone treatment.   It is interesting, although unexplained at the moment, that although the serum calcium level decreased with the infusion of phosphate, the excretion of calcium invariably increased.   It is possible that the infusion of phosphate either increased the filterable fraction of serum calcium or in some way interfered with calcium reabsorption.

Figures 1 and 2 summarize the data obtained in 48 clearance comparisons in each of the 2 dogs.   The quantity of phosphate reabsorbed by the tubules and excreted in the urine, expressed in mM/min., is plotted against the quantity of phosphate filtered through the glomeruli, likewise expressed in mM/min.   It is apparent that the rate of reabsorption of phosphate is essentially the same in the normal and in the parathormone-treated states in both dogs.

Eight additional experiments were performed on the same 2 dogs in a study of the effect of parathormone on the renal tubular reabsorption of calcium.   In 4 of the experiments parathormone was administered; in 4 no hormone was given, these latter serving as controls.   The method of administration of the hormone and all other experimental procedures were the same as in the experiments just described, except that the infusions contained no phosphate.

In order to calculate the quantity of calcium filtered through the glomeruli it is necessary to determine the fraction of the total serum calcium which is diffusible. To obtain this figure, 10 ml. of serum from each sample of blood were subjected to ultrafiltration under 150 lb. pressure of nitrogen through a cellophane membrane according to the method of Nicholas (18).   Consecutive 2 ml. portions of the filtrate as well as the original serum were analyzed for calcium by the methods used in the previous experiments.

Four typical experiments performed on the 2 dogs are illustrated in table 2. For each animal, the first 3 rows of figures constitute control observations; the last 3 rows constitute observations following the administration of parathormone. It is evident in these experiments as in those described above that there is no significant difference in serum phosphate level, in rate of reabsorption or in rate of excretion of phosphate in the control and in the parathyroid treated state.   All of our observations have been completely consistent in this respect.   However, we must point out that our experiments have all been performed from 20 to 23 hours after the initial dose and from 14 to 17 hours after the final dose of parathormone.   Thus any
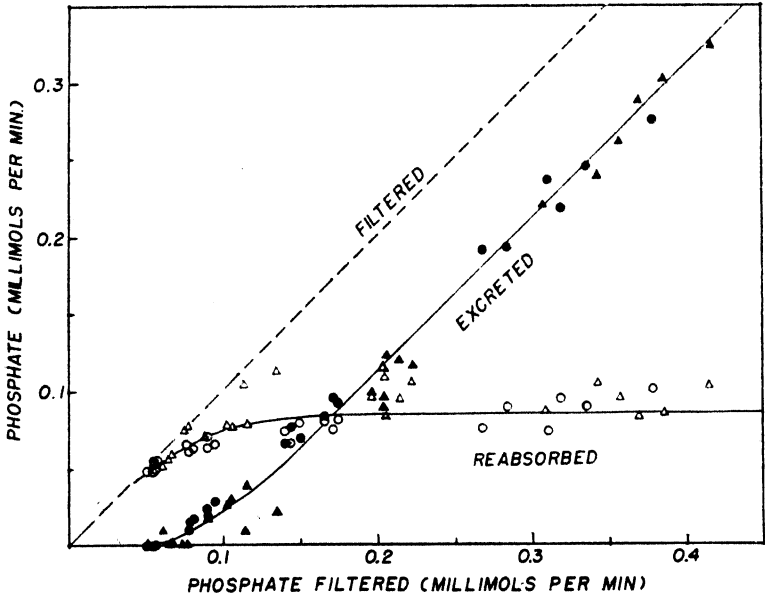
Fig. 1. RELATIONSHIP between quantity of inorganic phosphate filtered through glomeruli and quantities reabsorbed by renal tubules and excreted in the urine. *Dog P.* Circles, control observations; triangles, following parathormone.
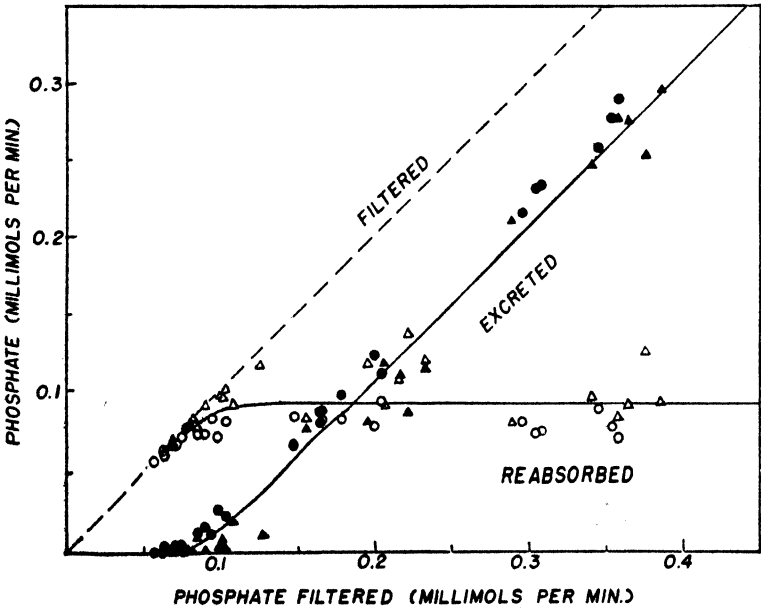
Fig. 2. RELATIONSHIP between the quantity of inorganic phosphate filtered through the glomeruli and the quantities reabsorbed by the renal tubules and excreted in the urine. *Dog R.* Circles, control observations; triangles, following parathormone.

transitory effect of the hormone on phosphate excretion or reabsorption would be missed.

In contrast it is evident from table 2 that the serum concentration of calcium and the rates of reabsorption and excretion of calcium were all significantly increased by parathormone administration. Thus during the control periods the serum concentration of total calcium averaged 6.9 mg. per cent in *dog R* and 7.7 mg. per cent in *dog P*;[5] that of filterable calcium averaged 4.1 mg. per cent in *dog R* and 4.7 mg. per cent in *dog P*. Following parathyroid hormone, serum total calcium increased

TABLE 2. EXPERIMENTS ILLUSTRATING RELATIONSHIP BETWEEN QUANTITIES OF CALCIUM FILTERED THROUGH GLOMERULI AND QUANTITIES REABSORBED BY TUBULES AND EXCRETED IN URINE IN A NORMAL DOG AND IN THE SAME ANIMAL FOLLOWING ADMINISTRATION OF PARATHORMONE.

GLOM. FILT. RATE	URINE FLOW	SERUM			PHOSPHATE			CALCIUM		
		Phosphate	Total calcium	Filtrate calcium	Filtered	Excreted	Re-absorbed	Filtered	Excreted	Re-absorbed
cc/min.	*cc/min.*	*mM/L.*	*mg%.*	*mg%.*	*mM/min.*	*mM/min.*	*mM/min.*	*mg/min.*	*mg/min.*	*mg/min.*
				Dog R; Control						
69.2	5.9	0.79	7.0	4.2	0.055	0.0005	0.055	2.91	0.004	2.90
70.2	7.1	0.71	6.8	4.1	0.050	0.0001	0.050	2.88	0.011	2.87
69.2	7.05	0.68	6.8	4.1	0.047	0.0001	0.047	2.84	0.029	2.81
				Dog R; Parathormone						
71.7	6.5	0.88	11.1	5.8	0.063	0.0009	0.062	4.16	0.150	4.01
66.5	4.9	0.85	11.0	6.8	0.057	0.0001	0.057	4.52	0.220	4.30
71.2	2.1	0.84	11.5	7.0	0.060	0.0001	0.060	4.99	0.350	4.64
				Dog P; Control						
60.9	3.05	0.64	7.0	4.6	0.039	0.0001	0.039	2.80	0.0009	2.80
63.6	1.65	0.63	7.8	4.8	0.040	0.0002	0.040	3.05	0.002	3.05
66.6	1.15	0.61	8.3	4.6	0.041	0.0002	0.041	3.06	0.004	3.06
				Dog P; Parathormone						
62.6	5.7	0.90	12.7	6.9	0.056	0.0002	0.056	4.32	0.250	4.07
60.9	3.25	0.90	12.3	6.5	0.055	0.0001	0.055	3.96	0.400	3.56
62.0	1.7	0.78	12.8	6.7	0.048	0.0002	0.048	4.15	0.320	3.83

to 11.2 and 12.6 mg. per cent, and filterable calcium to 6.5 and 6.7 mg. per cent respectively in the 2 animals.

It is apparent from the columns labelled calcium filtered, excreted and reabsorbed, that the increased delivery of calcium into the renal tubules, in consequence of the increase in serum concentration of filterable calcium, adequately accounts for the calcuria observed. Thus in *dog R* the quantity filtered rose from 2.88 to 4.56 mg/min., and in *dog P* from 2.97 to 4.14 mg/min. In *dog R* the quantity excreted rose from 0.015 to 0.240 mg/min., and in *dog P* from 0.002 to 0.323 mg/min. Since the increase in the quantity filtered greatly exceeded the increase in the quantity excreted, it is apparent that the quantity reabsorbed likewise increased, from 2.86 to

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[5] The cause of the low initial calcium in both of the control experiments is unknown. However the administration of parathormone in each instance increased serum calcium nearly 100%.

4.33 mg/min. in *dog R*, and from 2.90 to 3.82 mg/min. in *dog P*.[6] We feel that this increase in reabsorption is the consequence of the presentation of increased quantities of calcium to tubules whose reabsorptive capacities are unsaturated at ordinary plasma levels.

DISCUSSION

The experimental results presented above demonstrate that the administration of 600 U of parathormone causes no appreciable depression of the level of phosphate in the serum and no significant interference with the capacity of the renal tubules to reabsorb phosphate some 14 hours later.   Nevertheless at this time the serum concentration of calcium is maximally increased and calcium is excreted in the urine in large quantities.   Because the hypercalcemia and hypercalcuria persist for many hours, it is apparent that they cannot be causally related to any transient phosphaturia which immediately follows the administration of the hormone, but which subsides before the serum level of calcium and rate of excretion of calcium reach their maxima.   Our experiments are therefore consistent with the conclusion of Collip *et al.* (9) that the action of parathormone on the metabolism of calcium is independent of any direct influence it may have on the renal threshold for phosphorus.   Indeed we would go further in saying that at the time of greatest mobilization and urinary loss of calcium, the hormone exerts no effect on the reabsorption or excretion of phosphate by the kidney.

It is likewise evident that parathormone does not interfere with calcium reabsorption by the renal tubules.   Indeed there is greater reabsorption of calcium in the hyperparathyroid state than under normal conditions.   This increased reabsorption of calcium must mainly result from the fact that the kidney tubules are presented with greatly increased quantities of calcium in the glomerular filtrate.   Although more of the filtered calcium is reabsorbed, more also spills over into the urine to account for the evident hypercalcuria.

Monahan and Freeman (19) similarly believe that the parathyroid gland exerts an influence on calcium metabolism that is independent of the kidneys.   Thus they observed a decrease in the serum calcium concentration amounting to about 50 per cent in 72 hours in nephrectomized parathyroidectomized dogs, whereas no similar decrease was observed in nephrectomized controls.   The serum inorganic phosphate was markedly elevated in both groups.   Recently Ingalls, Donaldson and Albright (20) produced the characteristic bone lesions of hyperparathyroidism by the administration of parathormone to nephrectomized rats.   They are also of the opinion that their evidence supports the view that parathormone has a direct action on bone.

It is concluded from these and from our own studies that hypercalcuria and hypercalcemia are dependent on extrarenal actions of the hormone and not on any specific effect on the renal tubular reabsorption of either phosphorus or calcium.

SUMMARY

The effect of parathormone on the renal tubular reabsorption of inorganic phosphate has been studied over a range of plasma concentration of 0.9 to 5.48 mM/liter.

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[6] Comparable results were obtained in 4 additional experiments.

Under the conditions of our experiments, the rate of reabsorption of phosphate is essentially the same in the parathormone-treated and in the normal animal. The rate of reabsorption of calcium is greater following parathormone, for in consequence of increased plasma level of filterable calcium, increased quantities of calcium are presented to the tubules in the glomerular filtrate. Hypercalcemia and hypercalcuria produced by the administration of parathormone are dependent on extrarenal actions of the hormone in mobilizing calcium from body stores, not on any specific depression of the renal tubular reabsorption of either phosphorus or calcium.

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EFFECT OF ETHER AND PENTOBARBITAL ANESTHESIA ON CERVICAL LYMPH FLOW AND PROTEIN CONTENT IN THE CAT[1]

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THE volume of lymph flow and protein concentration of lymph in the dog have been shown by Polderman *et al.* (1) to be markedly affected by the type of anesthesia. Pentobarbital sodium caused a reduction in flow of about 50 per cent and ether caused an increase of about 50 per cent over the flow under local procaine anesthesia. It was the purpose of the present problem to study the effects of ether and pentobarbital sodium anesthesia on lymph flow and protein concentration of lymph in the cat and to compare these results with the effects noted in the dog by other workers.

METHOD

Fifteen cats weighing 2.4 to 4.4 kg. were used. These experiments were divided into four groups according to the anesthetic used or the order in which the anesthetics were administered. The first group consisted of the determination of the flow of lymph when the animal was first anesthetized with pentobarbital sodium and then, when the flow had reached a relatively steady state, with ether. The second group consisted of animals first anesthetized with ether followed by pentobarbital sodium when the flow had leveled off. The other two groups were control experiments and consisted of the determination of the effects of ether alone and of pentobarbital sodium alone. The animals were kept at a relatively light level of surgical anesthesia. The corneal reflex was used as an indicator of degree of anesthetization, except in a few isolated cases where it was absent. The respiratory rate then had to be relied upon to indicate the relative depth of anesthesia.

A 4 per cent solution of pentobarbital sodium was used. An initial dose of 40 mg/kg. was given intraperitoneally, and all subsequent doses needed to keep the level of anesthesia constant were given intravenously. A tracheal cannula was inserted and a small bottle partially filled with water was attached to the cannula. In this way the same amount of dead space was present under pentobarbital sodium as under ether.

When the cat was anesthetized with ether first, it was placed in a specially adapted can so constructed that ether vapor could be introduced. There was a small observation window in the top of the can. When the cat was anesthetized, it was transferred to the operating board and ether was administered through a cone using the drop method until a tracheal cannula could be inserted. A small bottle, through which the flow of ether vapor and air could be adjusted, was partially filled with ether and attached to the tracheal cannula.

Physiological saline (20 cc/kg.) was slowly administered to each cat·intravenously through a

femoral vein before lymph collection was begun, to insure adequate tissue hydration. The mean arterial pressure was recorded directly from a femoral artery using a mercury manometer. Blood samples were taken at regular intervals from the other femoral artery which had also been cannulated. Blood collected in capillary tubes was centrifuged and the hematocrit values determined from these samples. Serum protein determinations were done using a Zeiss dipping refractometer. Body temperatures, respiration rate and depth were noted.

The right and left cervical lymphatic ducts were isolated in all but a few cases when one or the other could not be found. Both lymphatics were cannulated in some cases. When only one lymphatic was cannulated, the other was tied off. A thin wire, dipped in dry heparin, was inserted in each cannula to prevent coagulation. In order to insure a continuous flow of lymph, a 'nodding preparation' was used (2).

The lymphatic cannulae were emptied at 5-minute intervals using a 'mosquito bill' pipette. All the lymph for a 15-minute period was collected in a single, previously weighed test tube. The protein concentration of the lymph was determined using a Zeiss dipping refractometer.

RESULTS

A summary of the data obtained is presented in tables 1 and 2. The values listed represent the average of the results secured during the period of one hour. The hour periods chosen were those during which the lymph flow had more or less leveled off. Whenever possible, two consecutive hours were used to note any immediate effects of changing the anesthetic agent. Figures 1-4 are graphs showing the course of four typical experiments, each one representing one type of experiment.

An examination of the average results reveals no marked changes in lymph flow when pentobarbital sodium anesthesia was followed by ether or when the reverse was the case. There was some variation in individual experiments, but there was a trend toward decreasing flow as the experiment progressed, regardless of the anesthetic employed. A slight rise in lymph protein content was found when pentobarbital sodium followed ether anesthesia, but no consistent change was noted when ether was introduced after pentobarbital sodium. The changes which occurred were of approximately the same magnitude as those found in the control experiments when ether or pentobarbital sodium alone was used for the duration of the experiment.

The hematocrit values decreased slightly when ether anesthesia was followed by pentobarbital sodium. This was also true of the serum protein values. On the other hand, when ether was introduced after pentobarbital sodium anesthesia, the effect was inconsistent; a rise in hematocrit occurred in two cases and a drop of the same magnitude occurred in the third. A majority of the serum protein values increased. The mean arterial pressure remained fairly constant in all experiments and the respiration rate showed no excessive variation.

The graphs show that, regardless of the anesthetic agent used at the outset of the experiment, there was a large initial flow of lymph. This was due to an accumulation of tissue fluid and lymph in the region of the head and neck during the period which preceded the beginning of the nodding motion. When the head movement was begun, the accumulated fluid was passively pushed into the cervical lymphatic ducts and out into the cannulae in relatively large amounts. However, in most cases the flow had dropped perceptibly by the end of an hour and had begun to level off during the second hour. Since the values obtained during the first hour are not indicative of the effect of the anesthetic used, they will be disregarded.

A certain amount of irregularity in lymph flow was encountered in all experiments. These variations cannot be accounted for by attributing them to changing the anesthetic used, since they occurred in control experiments as well.

TABLE 1. *A*. PENTOBARBITAL FOLLOWED BY ETHER ANESTHESIA. *B*. ETHER FOLLOWED BY PENTOBARBITAL ANESTHESIA

NO.	WT.	LYMPH		BLOOD			RESPIRATIONS/MIN.
		Flow	Protein	Hematocrit	Protein	Mean arterial pressure	
	kg.	mg/min.	%	%	%	mm. Hg	
A. Pentobarbital Anesthesia							
1♀	3.0	4.9	3.71		6.27	110	24
2♀	3.5	17.7	3.54		5.65	148	26
3♀	2.6	10.0	3.10		4.98	144	25
4♀	2.4	13.3	3.01		5.46	116	26
5♂	3.9	5.95	3.45	32.33	6.73	114	27
6♂	3.7	7.7	4.45	44.67	7.03	188	28
7♂	2.7	4.95	4.07	37.11	6.63	114	40
Average.............		9.2	3.62	38.04	6.11	133	28
A. Ether Anesthesia							
1		5.5	3.41		6.70	120	29
2		7.6	3.89		5.92	152	32
3		6.6	3.48		5.84	160	27
4		11.4	3.31		5.49	116	21
5		11.1	3.11	35.46	6.78	96	26
6		10.9	3.80	42.34	7.13	188	30
7		4.4	4.22	40.00	6.58	104	46
Average.............		8.2	3.60	39.27	6.35	134	30
B. Ether Anesthesia							
8♂	4.1	5.5	3.75	49.00	8.40	120	20
9♂	3.8	10.4	3.05	39.10	7.06	136	29
10♀	3.0	8.3	2.48	45.16	5.97	140	31
11♀	3.9	15.7	3.05	44.45	6.47	108	68
Average.............		9.97	3.08	44.43	6.98	126	37
B. Pentobarbital Anesthesia							
8		6.1	4.09	48.9	8.07	111	21
9		10.9	3.08	38.2	6.78	140	27
10		10.4	2.66	40.34	5.93	136	40
11		10.4	3.69	38.72	6.38	106	64
Average.............		9.5	3.38	41.54	6.79	123	38

The data obtained indicate that ether and pentobarbital sodium have no significant effect on lymph flow and protein concentration in the cat.
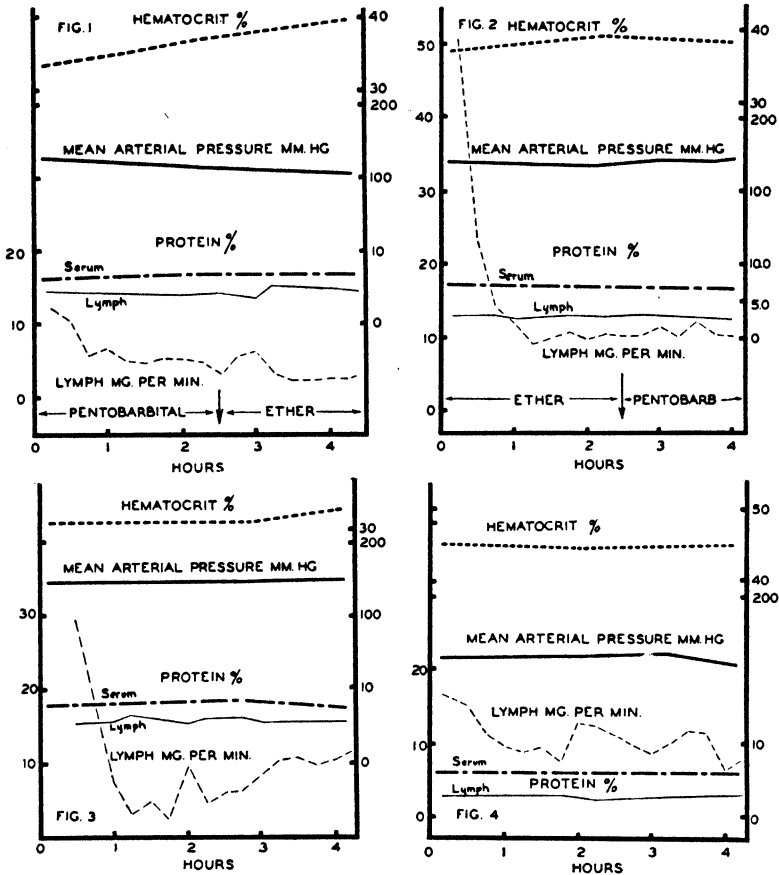
CHARTS ILLUSTRATING TYPICAL EXPERIMENTS. Arrows indicate the times at which anesthesia was changed.

Fig. 1. Pentobarbital sodium followed by ether anesthesia. This is one of the experiments in which the hematocrit increased. (*Cat 7*)

Fig. 2. Ether anesthesia followed by pentobarbital. (*Cat 9*)

Fig. 3. Pentobarbital sodium anesthesia only (*Cat 13*). Values for lymph protein and lymph were obtained by combining the yield from the right and left cervical lymphatic ducts.

Fig. 4. Ether anesthesia only (*Cat 15*).

DISCUSSION

In the light of the marked changes in lymph flow and protein content obtained by Polderman, McCarrell and Beecher (1) using ether and pentobarbital sodium anesthesia in the dog, the changes observed during these experiments on the cat cannot be called significant. No great increase in lymph flow under ether anesthesia or large decrease with pentobarbital sodium was noted. There is therefore apparently a species difference with regard to the effects of these two anesthetics on the lymph flow in the cat and dog. It has long been known that various anesthetic agents have different effects on the blood. It has been shown that ether causes hemoconcentration in dogs (3–10). Pentobarbital sodium causes hemodilution in

dogs (7, 8, 10, 11, 12).   There are indications that ether anesthesia decreases plasma volume in the human being (13, 14).

Hamlin and Gregersen (15) presented data which indicated that intravenous injection of nembutal caused an immediate increase in plasma volume of cats. Jarcho (10) found in cats that although nembutal anesthesia caused a marked decrease in hematocrit and plasma protein concentration, subsequent anesthetization of the cats with ether did not produce changes in blood concentration.   Conley (16) using ether, reported finding no change in plasma volume of cats.   If such a species difference can be shown to exist with regard to the effect of one anesthetic on the concentration of blood in these animals, it is conceivable that this difference will be reflected in the lymph flow.

TABLE 2.   CONTROL EXPERIMENTS

NO.	WT.	LYMPH		BLOOD			RESPIRA-TIONS/MIN.
		Flow	Protein	Hematocrit	Protein	Mean arterial pressure	
	kg.	mg/min.	%	%	%	mm. Hg	
		Pentobarbital Anesthesia only					
12♂	3.5	13.4	5.49		7.44	122	36
13♀	3.7	9.3[1]	5.73[1]	31.76	7.84	148	15
Average.............		11.4	5.61	31.76	7.64	135	26
		Ether Anesthesia only					
14♀	2.8	10.1	2.76	44.59	5.98	114	41
15♂	4.4	29.3	5.35	47.87	8.36	135	21
Average.............		19.7	4.06	46.23	7.17	125	31

[1] Combined flow from right and left cervical lymphatics.

SUMMARY

Ether anesthesia in comparison with pentobarbital sodium anesthesia had no significant effect on lymph flow and lymph protein concentration in the cat, thus suggesting a species difference between the cat and dog with respect to these anesthetics.

ADDENDUM

Since these experiments were performed, Smith *et al.* (17) have reported results obtained with regard to the immediate effects of ether and nembutal upon some blood components in the cat which indicate the immediate effect of ether on the blood is hemoconcentration and of nembutal, hemodilution.

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CHOLINESTERASE ACTIVITY IN NORMAL AND FEBRILE RABBIT BRAIN[1]

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IT IS generally assumed that in fever the temperature regulating center is set at a supranormal level and that active regulation occurs at this new level (1). It seems possible that this change in setting involves a metabolic change in the temperature regulating center. This change might involve an alteration in intracellular enzyme pattern which could be detected by enzyme assay. Technical difficulties precluded direct metabolic investigation of the cells of the hypothalamic centers. However, because of the qualitative similarity of the metabolic pattern in various parts of the central nervous system (2, 3), quantitative changes appearing in one region might be expected to be paralleled by changes in a similar direction in other regions. In view of current interest in the possibility that the acetylcholine-cholinesterase system is a factor in nerve function (4–6), we have compared the cholinesterase activity of homogenates prepared from brain tissue of normal and febrile rabbits. The results are reported in this paper.

Cholinesterase catalyses the hydrolysis of acetylcholine, with the formation of choline and acetic acid. The chemical and physiological properties of acetylcholine and the enzymes concerned with its synthesis and degradation have been reviewed by various workers (4–7). The manometric determination of cholinesterase is based on the displacement of CO_2 from a bicarbonate buffer by the acetic acid formed in the reaction. Thus hydrolysis of 1 mol of acetylcholine yields 1 mol of acetic acid, which in turn displaces 1 mol of CO_2 from the buffer medium (8–10).

METHODS

Cholinesterase was measured manometrically with the conventional Warburg technique. The suspension medium was Ringer's-bicarbonate buffer (calcium-free). Merck acetylcholine chloride was used as substrate in a final vessel concentration of 0.015 M and was made up in the suspension medium. The buffer solution was gassed for 20 minutes with a mixture containing 95 per cent N_2 – 5 per cent CO_2 before the vessels were filled. After the vessels had been filled with all necessary solutions, they were placed in a water bath which was maintained at a temperature of $39 \pm 0.01°C$. They were then gassed for 10 minutes with the 95 per cent N_2 – 5 per cent CO_2 mixture. After a 10-minute equilibration period, the acetylcholine solution was added from the sidearm of the vessels and readings were begun. Readings were made every 5 minutes for a 40-minute period. The rate of evolution of CO_2 during this time was constant.

Cholinesterase activity was determined for whole homogenate from rabbit brain and for the supernatant fraction of the homogenate. It has been shown previously that the supernatant fraction of the homogenates prepared in the Waring Blendor exhibits 35 to 45 per cent of the total activity of the whole homogenate (11), whereas a corresponding value of 10 to 20 per cent (11) is obtained when the Potter-Elvehjem homogenizer is used (12). Accordingly, all of our homogenates were prepared with the Waring Blendor. In order to maintain constant conditions throughout the experiments, all homogenates contained about 200 mg. fresh tissue per ml. Homogenates were prepared in Ringer's-bicarbonate buffer. A typical reaction flask mixture is given below:

Acetylcholine chloride, 0.15 M in Ringer's-bicarbonate buffer................ 0.15 ml.
Ringer's-bicarbonate buffer... 1.10 ml.
Homogenate or supernatant.. 0.25 ml.

Two rabbit brains were used for each experiment reported here. Of these, one served as a control and is hereafter referred to as the 'normal'. The other rabbit received an injection of 0.05 ml. typhoidparatyphoid triple vaccine[2] intravenously, and is hereafter referred to as the 'febrile'. Rectal temperature was checked from the time of injection throughout a period of one hour. A rise in rectal temperature of 1.0°C. was considered a satisfactory response. In most cases, the rise in rectal temperature amounted to 1.2 to 1.5°C. Exactly one hour after the time of vaccine injection, the 2 animals were killed by injection of air into the marginal ear vein. The brains were rapidly excised and placed in beakers surrounded by cracked ice. The homogenates were then prepared immediately in the cold room at 0.0°C.

RESULTS AND DISCUSSION

It is shown in table 1A that the values for cholinesterase activity in normal and in febrile rabbit brain homogenates are very similar. The difference in the mean values has been analysed statistically and has been shown to be insignificant (P = 0.26). Values are expressed both in terms of CO_2 production and of milligrams of acetylcholine hydrolyzed for convenience in comparison with other data in the literature. These values are calculated on the basis of 100 mg. fresh tissue per hour. Similar results for the supernatant fraction are shown in table 1B. Here, the agreement between normal and febrile animals is even more striking. It is readily apparent from inspection of the data that there is no significant difference between the two sets of values (P = 0.81). The results in table 1B are based on 1.0 ml. of a supernatant from a homogenate containing 100 mg. fresh tissue per ml. In this way it is possible to correlate the relative activity of the whole homogenate and of the supernatant fraction. It is interesting to note that the activity of the supernatant fraction amounts to about 40 per cent of that of the whole homogenate, which confirms the choice of the Waring Blendor when a high activity is desired in the supernatant.

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[2] Cutter Laboratories Typhoid-Paratyphoid Vaccine, containing 1000 million E. typhosa organisms per cc., 500 million S. paratyphi organisms per cc. and 500 million S. schottmuelleri organisms per cc.

The results presented here are in close agreement with those reported by other workers for normal rabbit brain cholinesterase activity. Nachmansohn and Feld (11) report that normal rabbit brain homogenate hydrolyzes 8.0 mg. acetylcholine per 100 mg. fresh tissue per hour. DuBois and Magnum (10) have found that normal rabbit brain homogenate produces 1224 microliters CO_2 per 100 mg. fresh tissue per hour, which is equivalent to the hydrolysis of 9.92 mg. acetylcholine per 100 mg. fresh tissue per hour. Our results of 9.07 mg. acetylcholine hydrolysed per 100 mg. fresh tissue per hour fall midway between these values. The results presented here represent, of course, the sum of cholinesterase activity in the various parts of the brain. Activity varies widely in different areas, as shown for rabbit

TABLE 1

	NORMAL		FEBRILE	
	µl. CO_2 evolved	mg. ACh hydrolysed	µl. CO_2 evolved	mg. ACh hydrolysed

PART 1A. Cholinesterase activity of whole homogenate from normal and febrile rabbit brain. (Values/100 mg. fresh tissue/hour.)   The symbols S.D. and N stand for the standard deviation and the number of runs respectively

	NORMAL		FEBRILE	
Mean....................	1119	9.07	1087	8.81
Range.................	982–1242	7.95–10.06	1039–1209	8.43–9.81
S.D.....................	79.58	0.645	49.40	0.403
N......................	12	12	12	12

PART 1B. Cholinesterase activity of supernatant fraction of homogenates from normal and febrile rabbit brain. (Values per 1.0 ml. supernatant from a 100 mg/ml. homogenate/hour.)

	NORMAL		FEBRILE	
Mean....................	435	3.52	433	3.51
Range.................	417–467	3.38–3.78	420–453	3.40–3.67
S.D.....................	14.77	0.118	12.37	0.101
N......................	12	12	12	12

brain by Nachmansohn and Feld (11).   For example, whole homogenate from the cerebral cortex of normal rabbit brain hydrolyzes 4.5 mg. acetylcholine per 100 mg. fresh tissue per hour, while a homogenate prepared from the nucleus caudatus is able to hydrolyze 31.6 mg. acetylcholine per 100 mg. fresh tissue per hour.

SUMMARY

Whole homogenate from normal and febrile rabbit brains hydrolyzes respectively 9.07 and 8.81 mg. of acetylcholine per 100.mg. fresh tissue per hour. These values are the means of twelve determinations. The difference between these means is not significant. The supernatant fraction of similar homogenates hydrolyzes respectively 3.52 and 3.51 mg. acetylcholine per 100 mg. fresh tissue per hour (mean of 12 determinations each). These means are also not significantly different. To the extent that these assays on whole brain can cast light upon processes mediating temperature regulation, they suggest that quantitative changes in cholinesterase assay are not associated with the 'thermostatic reset' which occurs in fever.

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DISTRIBUTION OF THE TWO FRACTIONS OF CHOLIN-ESTERASE IN HOMOGENATES OF PORTIONS OF THE DOG BRAIN[1]

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I T HAS been reported previously (1) that two fractions of specific cholinesterase are present in homogenates of mouse brain. One is found in the precipitate obtained by centrifuging the homogenate, and one remains in the supernatant fluid. The precipitate fraction is considerably more heat labile than is the supernatant fraction.

The present report is concerned with the total cholinesterase activity of homogenates of portions of the dog brain and the distribution of the two above fractions in the homogenates. In addition, the heat lability of the two fractions is reported.

PROCEDURE

Dogs were killed with pentobarbital sodium (*dog 1*) or with ether and chloroform (*dogs 2 and 3*). The brain and a portion of the cervical spinal cord were removed as quickly as possible. The brain of *dog 1* was dissected[2] immediately and the various portions were stored in closed containers in a CO_2 ice box until used. The brains of *dogs 2* and *3* were stored immediately in the CO_2 ice box. They were subsequently dissected, without thawing, and the various portions were returned to the ice box until used.

The tissue was homogenized, after thawing at room temperature in a fluid with the following composition: NaCl 0.15 M, $MgCl_2$ 0.04 M, and $NaHCO_3$ 0.025 M. The final substrate concentration was 0.015 M acetylcholine. The remainder of the procedure followed was that previously reported (1).

RESULTS

It will be seen in table 1 that when the supernatant fluid and precipitate fractions were heated at 53°C. for 60 minutes the average remaining cholinesterase activity of the supernatant fractions was 42.2 per cent of that present before heating, while the average remaining activity of the precipitate fractions was only 29.4 per cent of that present before heating. There was less remaining activity in each individual precipitate fraction than in the supernatant fluid fraction with the exception of the cerebellar peduncle and the internal capsule. These data are interpreted as evidence that the precipitate fraction of most portions of the dog brain is more heat labile than is the supernatant fraction.

In table 2 the cholinesterase activity of homogenates of the various portions of the brain which were studied will be found. It will be seen that the enzyme activity

varied considerably from one portion of the brain to another, the lowest being no detectable activity in the corpus callosum and the greatest activity being found in the caudate nucleus. It will be seen also that there is considerable variability among the various brain tissues in the per cent of the total activity which remains

TABLE 1. EFFECT OF HEATING UPON THE CHOLINESTERASE ACTIVITY OF SUPERNATANT FLUIDS AND PRECIPITATES OBTAINED FROM HOMOGENATES OF PORTIONS OF DOG BRAIN

($Q_{A \cdot Ch}$ = mg. of acetylcholine hydrolyzed/100 mg. wet weight/60 min.)

TISSUE	CONTROL $Q_{A \cdot Ch}$		PERECENTAGE OF CONTROL ACTIVITY REMAINING AFTER 60 MIN. HEATING AT 53°C.	
	Supernatant	Precipitate	Supernatant	Precipitate
Cerebellar nuclei..........................	2.52	6.13	38	21
Cerebellar peduncle......................	3.15	3.73	29	29
Cerebellar cortex.........................	3.08	8.91	55	13
Cerebellar cortex.........................	2.30	4.12	31	21
White matter.............................	0.44	0.56	70	54
Caudate nucleus..........................	8.59	34.99	22	12
Caudate nucleus..........................	6.46	16.82	33	21
Caudate nucleus[1]........................	7.92	17.77	20	10
Internal capsule..........................	1.15	1.70	60	60
Medulla..................................	1.76	3.27	57	36
Thalamus................................	1.68	3.21	49	46
Average...			42.2	29.4

[1] Heated at 54°C.

in the supernatant fluid fraction when the total homogenate is centrifuged. The supernatant fluid enzyme activity varies between an average of 27 per cent for the cerebellar cortex to a value of 55 per cent for the rostral portion of the cervical spinal cord.

DISCUSSION

With the exception of the caudate nucleus, the values reported here for the activity of the uncentrifuged homogenate agree generally with those reported by Nachmansohn (2). The values for the caudate nucleus are somewhat lower than those reported by Nachmansohn.

On the basis of the data presented in table 1, it appears that two fractions of cholinesterase are present in dog brain homogenate. The distribution of the two fractions between the supernatant fluid and the precipitate, obtained by centrifuging the homogenate, is considerably different from that reported for mouse brain (1) where the average values for supernatant fluid and precipitate were respectively 16.8 per cent and 82.9 per cent.

It will be seen in table 2 that as the estimated relative mass of nerve fibers in the tissues examined increases the general trend is for an increase in the per cent of the total cholinesterase activity which is found in the supernatant fluid from the homogenate. An exception to this is the corpus callosum, which had an enzyme

activity so low that it was not detectable with the procedure used here.   It is possible that the same relationship would be found in this tissue if a more sensitive procedure were used.   The relationship between the nerve fiber mass and the activity of the

TABLE 2.   CHOLINESTERASE ACTIVITY OF HOMOGENATES OF PORTIONS OF DOG BRAIN AND DISTRIBUTION OF ACTIVITY BETWEEN SUPERNATANT FLUID AND PRECIPITATE OBTAINED FROM THESE HOMOGENATES

DOG NO.	TISSUE	ESTIMATED % NERVE FIBERS	TOTAL $Q_{A \cdot Ch}$	SUPERNATANT FLUID ACTIVITY	PRECIPITATE ACTIVITY
				%	%
1	Cerebellar cortex	20	11.28	19	81
1	" "		12.73	24	70
2	" "		6.22	37	66
3	" "		8.47	29	72
Average...			9.68	27	72
1	Caudate nucleus	40	43.81	20	80
2	" "		23.38	28	72
Average...			33.59	24	76
1	Thalamus	40	5.59	44	64
2	"		5.01	34	64
Average...			5.30	39	64
1	Cerebellar medulla	70	8.33	31	74
3	Medulla	80	6.78	43	65
2	"		4.47	39	73
Average...			5.62	41	69
1	Spinal cord (C1–C3)	85	3.63	55	49
1	Internal capsule	100	2.59	44	66
1	White matter	100	0.93	47	60
1	Cerebellar peduncle	100	6.38	49	58
1	Corpus callosum	100	0		
2	" "		0		

supernatant fluid fraction is suggestive that this fraction may be more highly concentrated in the fiber or some portion of it and that its physiologic role may be related to this distribution.

SUMMARY

Dog brain homogenates have been shown to contain two fractions of cholinesterase similar to the previous finding with mouse brain.   The fraction present in the precipitate obtained by centrifuging the homogenate is more heat labile than is the supernatant fluid fraction.   The $Q_{A \cdot Ch}$ values for various portions of the dog

brain are as follows: caudate nucleus 33.6, cerebellar cortex 9.7, cerebellar medulla 8.3, cerebellar peduncle 6.4, medulla 5.6, thalamus 5.3, spinal cord 3.6, internal capsule 2.6, white matter 0.93, corpus callosum o.

In general, there is a direct relationship between the estimated relative mass of nerve fiber in the tissue and the activity of the supernatant fluid fraction of cholinesterase. With the exception of the corpus callosum, this relationship is suggestive that the supernatant fluid fraction may be more highly concentrated in the nerve fiber.

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THERMAL REACTIONS OF YOUNG ALBINO RATS TO INTRAPERITONEAL INJECTIONS OF ERGOTOXINE[1]

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ERGOTOXINE, administered intraperitoneally to albino rats over 30 days of age, has been found to be productive of hyperthermia if the animals are in a comfortably warm environment (1). The prevailing concept that ergotoxine has a central action has seemed to provide the most logical explanation for these phenomena.

Prior to the attainment of an age ranging between 18 and 30 days, albino rats are unable to maintain their body-temperatures when placed in a cold environment (2, 3). If the attainment of the ability to maintain a normal body temperature when exposed to adverse environmental situations depends upon the maturation of the hypothalamus (3) and if the thermal responses to ergotoxine are dependent upon hypothalamic stimulation, it seemed logical to hypothesize that pre-regulatory rats might fail to react to ergotoxine in the same manner as the older animals previously reported (1). The research presently reported was designed to test this hypothesis.

MATERIALS AND METHODS

Seven litters of Wistar strain albino rats have been used in the accumulation of the data specifically reported herein. A great many additional observations upon rats of comparable ages, but from other litters, have been made in connection with other studies; the thermal reactions of these animals have been similar, in all respects, to those presented.

Four rats from one litter (*2J*) were used as ergotoxine experimental animals and the remaining 3 served as controls. Ergotoxine ethanesulphonate,[2] dissolved in 6.25 per cent ethyl alcohol (1 mg. in 2 cc.), was administered intraperitoneally to the experimental rats in a dosage of 4.5 mg/kg. of body weight. Each animal received the drug on at least 8 separate occasions such that its thermal reactions were studied at intervals ranging from 12 to 53 days of life. Continuous temperature records were obtained concurrently from one experimental and one control animal by means of copper-constantan thermopiles and a two-channel Brown electronic potentiometer as previously described (1, 3).

The members of the other 6 litters were each given ergotoxine only once, at ages ranging from 12 to 32 days. Immediately after administration of the drug to the experimental animals in *litters 3N* and *3R* (fig. 3), each, together with a litter-mate

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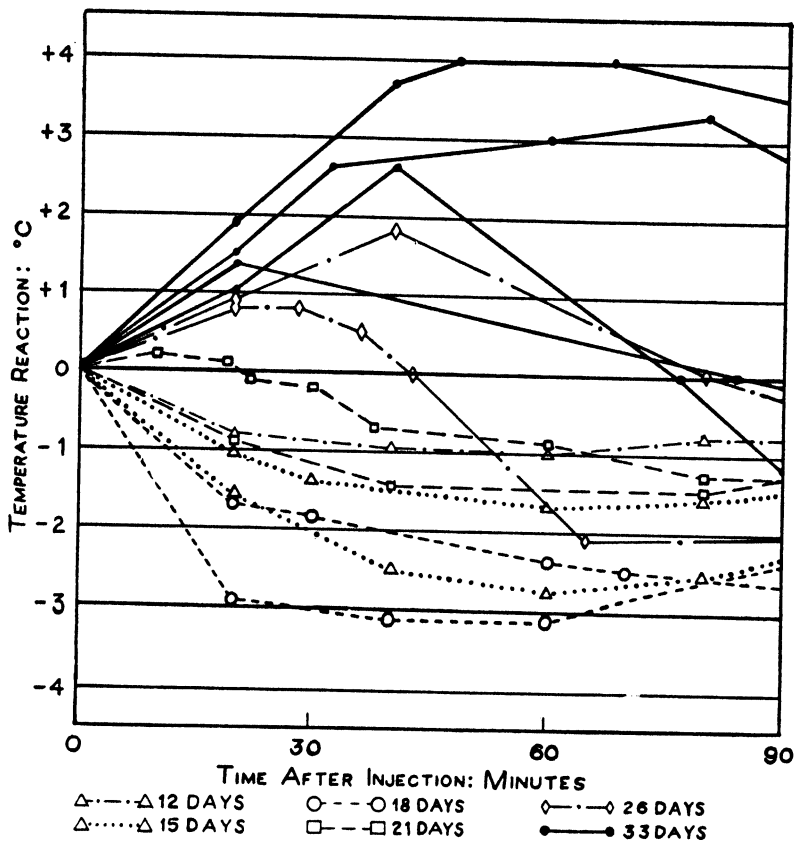
Fig. 1. THERMAL RESPONSES of litter-mate rats (*litter 2J*) given single intraperitoneal injections of ergotoxine ethanesulphonate (4.5 mg/kg.) on successive occasions and at the ages indicated.
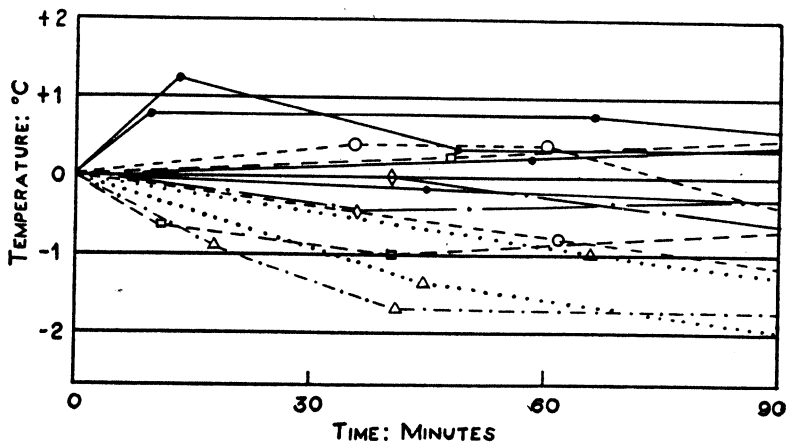
Fig. 2. TYPICAL TEMPERATURE RECORDS of litter-mate controls of experimental rats used in the compilation of figure 1. The legend accompanying figure 1 also applies to this figure.

control, was placed in a constant-temperature oven, set at 30°C., and the temperatures of both animals were recorded during a two-hour period. The temperature record of the ergotoxine-treated animal was then adjusted in such a manner as to compensate for minor variations in body temperature of the control which appeared to be attributable to the insertion of the thermopile into the colon. Control animals could not be studied concurrently with the experimental rats in the other 4 litters (*3Y*, *3Z*, *4G* and *4H*, fig. 3) since they were confined, during the period of observation of their ergotoxine reactions, within a metabolism chamber which was completely
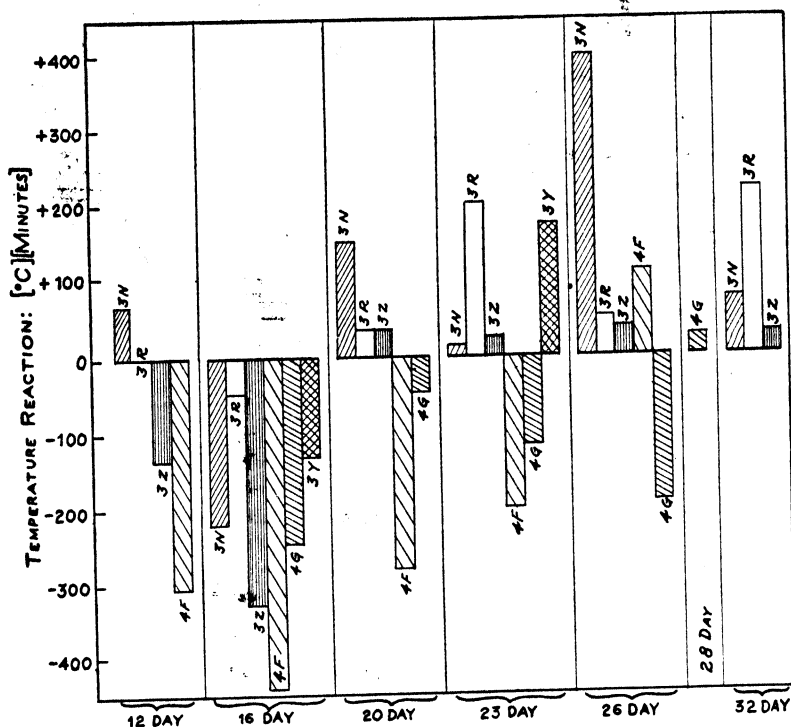
Fig. 3. THERMAL RESPONSES of individual members of 6 litters of rats given single injections of ergotoxine ethanesulphonate (4.5 mg/kg.) at the ages indicated. Hypothermic areas (in degree-minutes) cover a period of 2 hours. Hyperthermias (in degree-minutes) represent only the initial reaction.

submerged in a water bath; one channel of the potentiometer was utilized for a continuous record of the temperature of the chamber and this ranged between 29.5° and 30.5°C. The temperatures of litter-mates of the experimental rats in *litter 4G* were recorded, however, on the same days and under identical conditions.

RESULTS

Typical thermal reactions to ergotoxine at ages ranging from 12 to 33 days are shown in figure 1. All curves in figure 1 were obtained from the 4 experimental animals in *litter 2J* and they therefore depict, from 15 days on, the reactions of rats which had received one or more previous injections of the drug. The reactions of their litter-mate controls to simple confinement and insertion of the thermopile are indi-

cated in figure 2 and are obviously much less pronounced than the reactions in ergotoxine-treated animals. The 12- and 18-day rats exhibited hypothermia in response to ergotoxine which was more pronounced at 18 than at 12 days. Hyperthermic responses were well developed at 26 days of age and pronounced at 33 days. Beyond the 33rd day the responses continued to be of considerable magnitude and on the side of hyperthermia.

Rats given single injections of ergotoxine at ages ranging from 12 to 32 days responded in the same manner as those receiving successive injections at corresponding ages. The thermal reactions of the members of 6 litters are plotted as degree-minutes in figure 3. With the exception of the 12-day rat from *litter 3N*, all the 12- and 16-day animals exhibited hypothermic responses.

Hyperthermia appeared on the 20th day in *litters 3N, 3R,* and *3Z,* on the 23rd day in *litter 3Y,* on the 26th day in *litter 4F,* and on the 28th day in *litter 4G.* The temperatures of all the animals in these 6 litters were recorded for 2 hours, but the hyperthermic areas, as plotted in figure 3, include only the initial responses. Hypothermias, which regularly follow the primary hyperthermic reactions, were not summed with the latter. As was true in individual members of *litter 2J* (fig. 1), the duration of the initial response at a given age varied considerably from litter to litter.

Litter-mate controls from *litter 4G,* studied under identical conditions except that they did not receive ergotoxine showed only minor fluctuations in temperature.

DISCUSSION

The fact that ergotoxine failed to elicit hyperthermia in young (pre-regulatory) rats (with one exception) appears to be related to the inability of animals of the same strain and within the same age range to maintain their body-temperatures when placed in a cold environment (3). If ergotoxine hyperthermia is due to direct action of the drug on the hypothalamus (4, 5), its failure to appear in young rats seems to indicate a lack of maturity of this area of the brain and the results presented above may be considered as strongly supporting our previous contention that the attainment of regulatory ability is dependent upon maturation of the hypothalamus and its descending connections (3).

The hypothermic reactions to ergotoxine of the young rats were unexpected and are not easily explained. It is possible that they are due in part, at least, to peripheral vasodilatation associated with sympathetic reversal as described by Dale (6) and that the hypothermia in 16- and 18-day animals is more pronounced than in those 12 days of age because of a greater degree of maturity in the peripheral sympathetic mechanisms of the older rats. If this hypothesis is accepted, it is necessary to assume further that, upon maturation of the central regulatory mechanism in the hypothalamus, the more primitive peripheral response to ergotoxine is superseded by it.

The hyperthermic responses to ergotoxine are usually accompanied by considerable muscular activity and the hypothermic response (in the immature animals) by an almost anesthetic quiescence. It is worthy of note, however, that the 26-day rat in *litter 4G* displayed increased muscular activity in the presence of hypothermia; this, together with other similar observations, suggests that the hyperthermic reactions are only partially due to the effect of ergotoxine on muscular activity.

SUMMARY

The hypothesis that thermal responses to ergotoxine of rats in the pre-regulatory age range might differ from those of regulatory animals has been experimentally verified.   It has been found that rats 12 to 18 days of age usually react to ergotoxine with varying degrees of hypothermia as contrasted to the reactions of their older litter-mates (20–30 days), which, under identical environmental conditions, are almost uniformly on the side of hyperthermia.   Variations from litter to litter, so far as the age at which reversal of the response occurs, are common.   Such variations have also been observed with regard to the age at which attainment of the ability to regulate body temperature occurs.   The age range of reversal of the ergotoxine reaction (from hypo- to hyperthermia) and that at which regulatory ability is attained closely coincide.   These observations lead to the conclusion that both phenomena depend upon the maturation of the hypothalamus.

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EXPERIMENTAL MYOTONIA AND REPETITIVE PHENOMENA: THE VERATRINIC EFFECTS OF 2,4-DICHLORPHENOXYACETATE (2,4-D) IN THE RAT[1]

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THE profound botanical effects of certain substituted phenoxyacetic acids have been described by several investigators of plant growth (1, 2). A familiar example of these growth-regulating agents is 2,4-dichlorphenoxyacetic acid (2,4-D); but over one thousand compounds, directly or remotely related, have been examined and many were found to exert similar effects on plants (3).

Certain extraordinary effects of 2,4-D were noted in representative mammals by Bucher (4). Most striking was the development of muscular rigidity which appeared to be myotonic in character and was reported to be so established by myographic and electromyographic analysis.

This communication reports certain effects of 2,4-D on the neuromuscular system of the rat mimicking the myotonia which occurs spontaneously in man (5) and in the goat (6–8). These effects were indistinguishable from those produced by a large and apparently unrelated group of chemical and physical stimulating agents.

METHODS

The rats were of both Whelan and Sherman strains, weighing 140 to 400 g. The triceps surae and sciatic nerve and its tibial branch were studied exclusively. Anesthesia was induced by sodium pentobarbital (Nembutal). Skin and tissue overlying the sides of the distal end of the femur were split for approximately 5 mm. The underlying femur was fixed rigidly by a clamp designed on the principle of a micrometer; the shaft of the femur was held between the advancing spindle and anvil, and the sleeve was fixed to the myograph frame. A short incision over the heel permitted separation of the tip of the calcaneus with its attached tendon. The muscle, when prepared for recording, enjoyed an undisturbed blood supply and was covered with intact skin. The tendon was transfixed by and secured to a short hook of piano wire (No. 20 B. & S. gage) which was attached to a cylinder of bakelite for insulation. The upper end of the bakelite cylinder was screwed to the armature pin of a Statham YE-48-600 strain gage.[2] The strain gage was mounted rigidly to the main column of a Brown-Schuster myograph. This type of strain gage is particularly adapted

for isometric recording; the movement of the pin under a full load of 1500 g. is limited to 0.04 mm. The bridge signal was fed through a direct-coupled amplifier to a cathode ray oscilloscope for photographic recording. The bridge output was linear through full-scale deflection. Resting tension of the muscle was maintained at a constant value throughout any one experiment, but varied from one experiment to another between 75 and 200 g.

For indirect stimulation the sciatic nerve was crushed and tied proximally. The distal segment was placed in a plastic insulating bed containing two silver stimulating electrodes. Direct stimulation was effected through steel needles placed at opposite ends of the muscle. Stimuli, which were supramaximal in intensity, were presented as square waves generated by a trigger circuit, the duration of which was controlled by a variable resistance-capacitance circuit.

Nerve action potentials were recorded through a Sherrington electrode from the tibial nerve isolated peripherally. Stimulation was effected at the pelvic end of the sciatic nerve which was isolated centrally.

Electromyograms were recorded through steel needles insulated to within 1 to 2 mm. of their tips and placed in the belly of the muscle approximately 5 mm. apart. The potentials were fed through a condenser-coupled amplifier to the cathode ray oscilloscope and recorded photographically. When the needles were inserted shortly after section of the sciatic nerve, spontaneous random spike potentials were observed. These spikes lessened in frequency and then disappeared in the course of 10 to 15 minutes. No studies were carried out until spontaneous activity had subsided.

Intra-arterial injection was made through a small cannula tied into the contra-lateral iliac artery and directed centrally. The crural artery of the leg under study was ligated. The aorta was occluded during injection by drawing up on a loose ligature placed above the bifurcation. The injection volume was 0.15 ml. This preparation preserved normal blood supply to the muscle under study but is under continuing development to attain further restriction of the injection to the triceps surae. The small size of the rat's peripheral vessels enabled the use of a simple high-frequency desiccating apparatus ('Hyfrecator', Bircher) to coagulate vessels and, thus, to reduce bleeding.

When required, denervation of the triceps surae was performed 10 days before recording by aseptic section of the sciatic nerve.

Concentrations of potassium in serum and muscle were determined in control rats of the same weight in whom equivalent amounts of KCl had been injected intra-peritoneally (i-p). Analyses were made in a modified Berry-Chappell-Barnes in-ternal standard flame photometer (9, 10).

Prior to administration of curare the trachea was cannulated and artificial res-piration provided by a pump.

The concentrations of the agents used and their dosages follow:

1) Sodium pentobarbital, 25 mg/ml., was administered i-p in doses of 40 to 50 mg/kg. body weight.

2) Sodium 2,4-dichlorphenoxyacetate monohydrate (Baker) was dissolved in water in a con-centration of 50 mg/ml., and the *p*H was adjusted to 7.4 with dilute HCl for i-p injection in doses of 100 to 250 mg/kg.

3) *d*-Tubocurarine chloride[3] was injected i-p in doses of 1.5 mg/kg. This is equivalent approximately to one unit of curare per animal and produced total paralysis of all skeletal muscle.

4) KCl was injected i-p as a 20 mg/ml. (270 mEq/l.) aqueous solution. Doses were 400 mg/kg. (5.4 mEq.).

5) Quinine dihydrochloride was injected intramuscularly in aqueous solution of 45 mg/ml. and in doses of 150 mg/kg.

6) Disodium d-l-α-tocopheryl phosphate[4] was injected i-p in an aqueous solution of 100 mg/ml. in doses of 1 g/kg.

7) Magnesium sulfate was injected i-p in doses of 250 mg/kg.

8) Calcium gluconate was injected i-p in doses of 100 mg/kg.

RESULTS

Our observations are in full agreement with those of Bucher on the behavior of the rat which has received 2,4-D (4). In both conscious and anesthetised rats full development of myotonia appeared some 30 to 45 minutes after i-p injection and lasted for hours. The injection of small amounts (2 mg.) of 2,4-D into the vascular tree was followed by extreme generalised myotonia in two minutes. Even under deep anesthesia and full curarisation, the resistance to passive motion of the extremities was appreciably enhanced.

Primary effects of 2,4-D on muscle function are illustrated in figures 1 and 2. Increase in tension developed by an isometric twitch, in response to a single supramaximal stimulus to the nerve, was of the order of 25 to 30 per cent; duration of twitch until half relaxation was greatly prolonged (figs. 1A and 2A).

The electromyogram of normal muscle stimulated by a single shock to the nerve is a simple diphasic deflection (fig. 1B). When myotonia had developed after injection of 2,4-D the response became repetitive. The rapidly recurring, brief potential changes were less than the initial spike, and occasionally they did not appear until after a short period of electrical silence, 10 to 50 msec., following the initial spike. On one occasion the silence persisted for 600 msec. (fig. 3C). In some records the regular rhythmicity of the repetitive response suggested that a single motor unit was firing directly under the recording electrodes. The duration of repetition was variable, ranging from 100 msec. to 6 sec.

Prolongation of twitch and repetitive firing decreased rapidly with repeated stimulation; at a stimulus rate of 12 per min. myotonic features diminished rapidly during the first five or six consecutive single volleys (fig. 1A, B). Rest for 10 minutes resulted in complete return of the myotonic response. The same changes were noted following stimulation by a pair of nerve volleys delivered at short intervals.

Myotonia in man and goat is characterised by exquisite sensitivity of muscle to mechanical stimuli. 2,4-D produced in the rat this same explosive electrical response to tapping of the muscle or tendon and also to the insertion or movement of the recording electrodes (fig. 2).

The site of development of the myotonic response to 2,4-D was delimited partially by injection of sufficient *d*-tubocurarine chloride to block completely any muscle

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[3] Generously provided by E. R. Squibb and Sons, New York, N. Y., and by Abbott Laboratories, North Chicago, Ill.

[4] Generously provided by Hoffman-LaRoche, Nutley, N. J.

response to indirect stimulation. Under these conditions, direct electrical or mechanical stimulation of curarized muscle produced increased twitch tension, protracted relaxation time and repetitive response indistinguishable from that observed in non-curarized muscle (fig. 3). The triceps surae, denervated 10 days before examination, also developed myotonia in response to 2,4-D despite obvious atrophy.
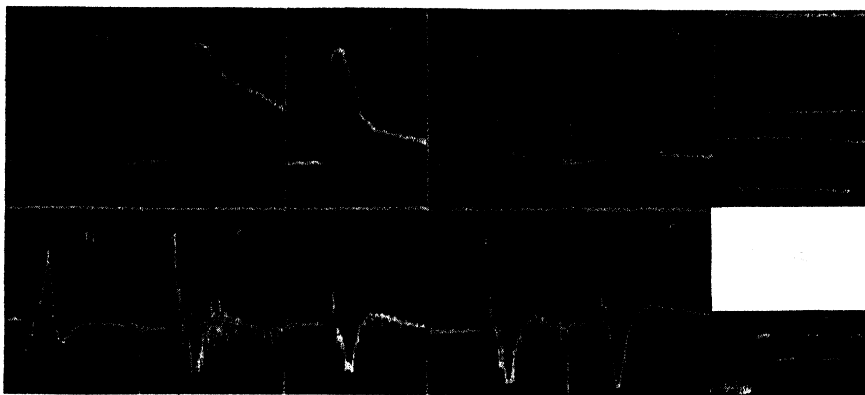
Fig. 1. INDIRECT STIMULATION OF THE TRICEPS SURAE BY THE SCIATIC NERVE. *A*. 1. Norma myogram. 2–5. Consecutive myograms, 120 min. after 40 mg. of 2,4-D, in response to stimul delivered at a rate of 12/min., illustrating decreased duration and the phenomenon of 'warm-up'. 6. Time: 50 msec. 7. Tension: 200 g. *B*. 1. Normal electromyogram. 2–5. Consecutive records as in *A*. 6. Voltage: 200 μV. 7. Time: 50 msec.
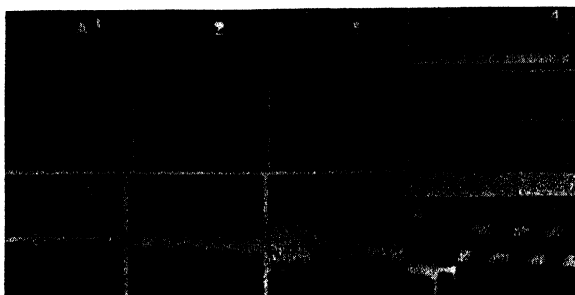
Fig. 2. INDIRECT STIMULATION AND MECHANICAL TAPPING. *A*. 1. Normal myogram. 2. 30 min. after 30 mg. 2,4-D. 3. 39 min. after 60 mg. KCl (same preparation as 2). 4. Time: 50 msec. 5. Lower line: zero tension; middle line: resting tension of 143 g.; upper line: 200 g. *B*. Electromyogram in response to a brisk tap on tendon. 1. Normal. 2 & 3. Corresponds to A2 and 3. 4. Voltage: 1 μV. 5. Time: 50 msec.

The familiar simple diphasic electrogram of nerve stimulated by a brief single shock is recorded in figure 4 (11). One hour after administration of 2,4-D a similar single stimulus evoked a volley of potentials which arose in the nerve proper, since it had been isolated from both central and peripheral structures.

Certain agents which modify spontaneous myotonia were studied for their effects on the myotonia produced by 2,4-D. *Potassium*, 5.4 mEq/kg. i-p, elevated the serum concentration from a normal level of 5 mEq/l. to 10 to 14 mEq/l. in 20 to 30
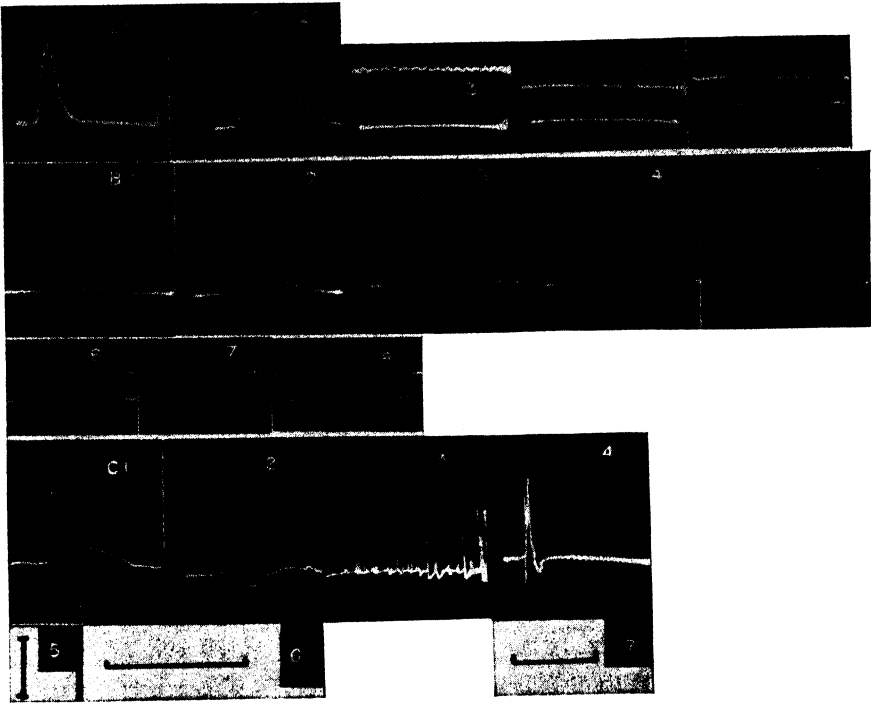
Fig. 3. COMPLETELY CURARIZED PREPARATION; DIRECT STIMULATION.　A. 1. Normal myogram 2. 45 min. after 40 mg. 2,4-D.　3. Tension: 200 g.　4. Resting tension: 125 g.　5. Time: 50 msec. B. 1. Normal myogram.　2. 45 min. after 40 mg. 2,4-D.　3. 11 min. after 20 mg. quinine.　4. 5 min. after additional 20 mg. quinine.　5. 10 min. after 4.　6. Tension: 200 g.　7. Resting tension: 200 g.　8. Time: 50 msec.　C. 1. Normal electromyogram.　2 & 3.　Consecutive sweeps 40 min. after 30 mg. 2,4-D, illustrating an unusually prolonged period of silence (600 msec.).　4. 5 min. after 40 mg. quinine (irregularity of trace is owing to 120 cycle interference).　5. Voltage: 1 μV.　6. Time: 50 msec. (referring to 1–3).　7. Time: 50 msec. (referring to 4).
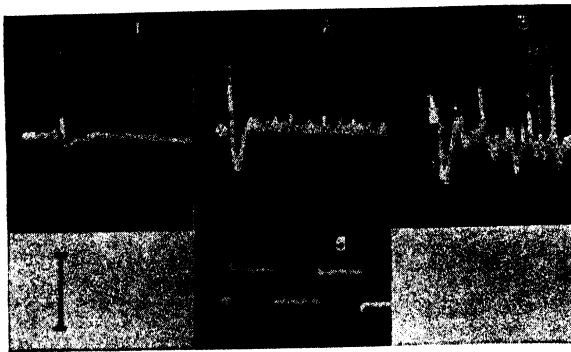
Fig. 4. DIPHASIC ACTION POTENTIAL FROM TIBIAL NERVE.　1. Normal.　2. 15 min. after 30 mg. 2,4-D.　3. 60 min. after 2,4-D.　4. Voltage: 200 μV.　5. Time: 50 msec.

minutes.　This increase in serum concentration presumably was reflected rapidly in the interstitial fluid surrounding the muscle cells, but analyses of the total muscle itself revealed no significant increase in total K content.　We have confirmed

Walker's findings that in the normal rat this distortion of K^+ relationships resulted in a heightened and lengthened tension response to both direct and indirect stimulation and in repetitive firing of the muscle in response to a single stimulus (12). The administration of KCl to a rat in full course of response to 2,4-D produced a further potentiation of the myotonic response and in degree of repetitive firing (fig. 2B).

Increased sensitivity to *acetylcholine* was demonstrated by injecting 100 μg. intra-arterially (11). In the preparation described here, this injection elicited a few small scattered spike potentials in the electromyogram. After treatment with 2,4-D the same amount of acetylcholine evoked an explosive and prolonged burst of electrical activity.

Quinine has been shown to reduce or obliterate the myotonic response occurring spontaneously in man (13, 14) and goat (6, 8). The same effect was produced on myotonia induced in the rat by 2,4-D. The myogram revealed a lowering of tension following administration of quinine (fig. 3B). There are certain characteristics of this response which warrant noting. First, tension developed after the second dose of quinine fell to 54 per cent of the control twitch; and second, the duration of the twitch was not reduced. The effect of quinine on the responses induced by 2,4-D is shown in figure 3C where the characteristic repetitive firing of the muscle was obliterated.

Alpha-tocopheryl phosphate, when injected i-p in relatively large doses into normal rats, produces profound generalized effects: somnolence, ataxia, muscular flaccidity, coma and, occasionally, death following convulsions (15). When administered per-orally to two patients with myotonic dystrophy a suggestive decrease in spontaneous myotonia was observed (16). Injection of α-tocopheryl phosphate in the rat, which had received 2,-4D, obliterated the evidences of myotonia as effectively as did quinine.

Similar suppression of repetitive response followed injection of *magnesium* and *calcium*.

DISCUSSION

The neuromuscular apparatus of the rat, treated with 2,4-D, mimics faithfully the characteristics of myotonia occurring spontaneously in man and goat. In both circumstances, muscle exhibits exquisite sensitivity and repetitive response to several stimulating agents: motor nerve volley and direct stimulation, tapping, insertion of needle electrodes, acetylcholine and K^+ (6). Furthermore, in both instances, repeated stimulation ('warm-up'), quinine, Mg^{++} and Ca^{++} diminish and obliterate the repetitive phenomenon. Alpha-tocopheryl phosphate now may be added to the list of agents inhibiting repetitive responses in both spontaneous and induced myotonia.

These effects of 2,4-D, producing a myotonic reaction so reminiscent of the spontaneously occurring forms, are indistinguishable, likewise, from the consequences of treatment with a variety of substances; e.g., the 'veratrine' alkaloids derived from *Veratrum* and *Schoenocaulon*, aldehydes, tetraethyl ammonium ions, phenanthrene-9-carboxylic acid and substituents, and dihydronaphthacridine carbonic acid

(Tetrophan) (17). To this heterogeneous group we have added recently pentamethylene tetrazol (Metrazol) (18).

Many, and perhaps all, of these diverse veratrinic substances, however, produce the same repetitive response in nerve that they produce in muscle. It has not been established in spontaneous myotonia whether or not this repetitive phenomenon occurs in nerve as well as in muscle; nevertheless, some observations have suggested that myotonia in man is accompanied by functional changes not limited to muscle fiber proper (19). Indeed, it is reasonable to view repetition as a stereotyped response of excitable tissue (nerve, neuromuscular junction and muscle) to many unrelated alteratives which may play rôles of differing intensity on all excitable structures. A tentative exploration of this possibility might begin by examining the circumstances in which repetition has been observed.

In order to limit the analysis, only those instances of repetitive response to single stimuli have been considered; obviously, this limitation excluded other important changes in excitability manifested by spontaneous firing or by measurable changes in pre-discharge conditions, such as threshold, resting potential, recovery cycle etc. (20, 21). The appearance in excitable tissue of repetitive, rhythmic responses to a single stimulus occurs under the most diverse of circumstances, as for example: *a*) increased external hydrostatic pressure (22); *b*) stimulation by constant rectangular currents (23); *c*) increased external concentration of H^+ (20, 24), K^+ (12), acetate, lactate, citrate and oxalate (24), Ba^{++} and guanidine (25), adenosine triphosphate (26), DDT and quinoline (27); *d*) decreased external concentration of Ca^{++} (28); *e*) inhibition of cholinesterase activity at the neuromuscular junction by eserine (11), neostigmine (29) and DFP (30); *f*) tetanus toxin affecting the neuromuscular junction (31); *g*) treatment with that heterogeneous group of substances which produce veratrinic effects (17); and *h*) spontaneous myotonia in man and goat.

In the face of our fragmentary knowledge of processes subserving excitability, the incongruity of those conditions and agents producing repetitiveness makes it extremely difficult to apply any unifying concept which can explain all of the observations noted above. An example of the difficulties encountered in a simple approach is contained in an attempt to analyse the phenomenon of repetitiveness in terms of distortions of cationic *milieu*. Preservation of totally normal function depends on maintenance of concentrations of Na^+, K^+ and Ca^{++} within certain limits. Could these many agents act by disturbing the required cationic patterns? Simple inspection of the structure of the veratrinic agents reveals their variation through almost full scale with respect to chemical activity, steric and physical characteristics; some are potential metal sequestrants or precipitants, others totally inactive. Lack of any common characteristic is emphasized not to imply that a unitary pattern of action is non-existent but to stress the necessity for fitting many divergent observations into any proposed scheme.

It seems likely that these many different forces and agents may act at different loci in the complicated train of events responsible for the smoothly integrated flow of energy which maintains normal excitability. Only vague clues exist to suggest certain possible sites of action.

For example Lorente de Nó, from an elaborate analysis in frog nerve, suggests

that, "Veratrine by reducing the speed of or blocking some late step in the chain of oxidative processes interferes with the maintenance of the membrane potential. . . ." (24). From a study of plants and micro-organisms comes suggestive evidence that 2,4-D distorts mechanisms involved in the transfer of oxygen (32, 33). Compatible with, but not proving, this suggestion is the suppressive action of Mg^{++} on veratrinic phenomena (24) and the similar action of α-tocopheryl phosphate which is so potently antioxidative as well as antiproteolytic (15).

Models of excitability or energy transfer expressed in terms of chemical or physical structure, enzymatic activity, surface activity or membrane potential, may explain some or many of the observations; but none, as yet, has been offered in satisfactory interpretation of all the data.

The phenomenon of the silent period intervening between the normal single spike potential and the outburst of repetitive spikes following treatment with 2,4-D is the same as that described by Eichler in the frog treated with small amounts of 'veratrine' (34). A speculative interpretation of this phenomenon might assume exponential decay of facilitating and depressing processes initiated simultaneously by the stimulus (35). If under the observed circumstances the depressing process decayed so rapidly that the facilitating process remained unopposed, then a burst of activity might be released. The data available do not permit a more specific analysis.

SUMMARY

2,4-dichlorphenoxyacetate produces in the neuromuscular apparatus of the rat a veratrinic response marked by repetitive response to single stimuli in muscle and nerve. This results in increased 'twitch' tension and prolonged 'twitch' duration. The repetitive responses and their sequelae are accentuated by K^+ and acetylcholine and are obliterated by activity, quinine, Mg^{++}, Ca^{++} and α-tocopheryl phosphate. These phenomena are indistinguishable from those occurring in the spontaneous myotonia of man and goat, and also in response to several apparently unrelated chemical, physical and electrical agents.

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EFFECT OF MECHANICAL VIBRATION ON THE PATELLAR REFLEX OF THE CAT[1]

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IN 1938 Coermann (1) surveyed some of the physiological effects of the exposure of man to mechanical vibration in the frequency range 20 to 1000 cps. One of the few positive results obtained was that, during the operation of a vibrating platform on which a human subject was seated, it was difficult or impossible to obtain the patellar reflex. This phenomenon was further investigated by Loeckle (2). Briefly, the latter found that the application of a vibrating rod to the skin area over the course of the femoral artery, vein and nerve of a human subject produced abolition or reduction of the reflex, whereas if the rod was applied directly to the quadriceps muscle, no inhibitory effect was found. Further, in one experiment on an anesthetized cat, he found that application of the vibrator to the femoral artery which had been lifted free from the surrounding tissues for a distance of a few cm. abolished the reflex. When the artery was stripped of its adventitial layer, the inhibitory effect vanished. In addition, he studied an individual on whom a unilateral lumbar sympathectomy had been performed one year before. On the sympathectomized side only it was found impossible to inhibit the patellar reflex by vibration. He used frequencies from 30 to 100 cps. and amplitudes up to about one mm. Loeckle naturally concluded that the inhibition was mediated by the sympathetic fibers of the periarterial plexus and the sympathetic ganglionic chain.

Echlin and Fessard (3) applied a vibrating tuning fork (85–530 cps.) or a vibrating steel strip (5–32 cps.) to a muscle or its bony support, and recorded nerve action potentials. The potentials tended to be synchronized with the vibrating stimulus. Sommer (4) obtained electromyograms from the biceps of the vibrated arm of a man (37 cps.). These potentials were also synchronized with the vibration. On the other hand, Loeckle's electromyograms of the human quadriceps showed no such impulses.

In view of these observations, the rôle of the autonomic nervous system in mediating the reflex inhibition seems doubtful. The intrinsic interest of these phenomena, together with their practical importance in relation to human exposure to vibration, justified further experimental work.

MATERIAL AND METHODS

Successful experiments were carried out on 7 cats. Two of these experiments also involved decerebration and two more, lumbar sympathectomies. The animals were anesthetized with ether and the quadriceps femoris was exposed together with its tendon, nerve and vascular supply, using

great care not to damage these structures. The nerve supply to antagonistic muscle groups was severed.

Mechanical vibration was applied with a permanent magnet loud speaker from which the cone had been removed and to which a rod 6 inches long by ¼-inch diameter had been fixed. The device was driven by an audio oscillator and a 15-watt public address system amplifier. It was mounted on an adjustable stand over the animal operating board so that it could be lowered vertically into contact with the part to be stimulated. The vibrator also could be applied manually in other directions. Observations of the wave form of the vibrator indicated roughly 10 to 20 per cent harmonic content. Frequencies of 10 to 600 cycles per second were used. The maximum amplitude obtainable at the lower frequencies was about 1 millimeter, with less at the higher frequencies. For most purposes, 100 cycles and about 1-millimeter amplitude were used. The patellar reflex was elicited by regular, controlled tapping of the tendon.

RESULTS

When the vibrator was applied directly to the belly of the muscle or to the tendon or the femur, the knee jerk was much reduced or was abolished entirely. The effect appeared immediately upon applying the vibrator and disappeared immediately on removing it. The muscle was often seen to undergo a slight contraction which persisted as long as the vibrator was applied. Application of the vibrator to the artery *in situ* occasionally produced a very slight diminution of the reflex. However, when the artery was lifted free from the underlying structures for a distance of a centimeter or so, no inhibitory effects could be obtained.

Two cats were also decerebrated and further prepared to observe contraction of the quadriceps by elicitation of the crossed extensor reflex (stimulation of the central cut end of the opposite sciatic nerve). During application of vibration, with the patellar reflex inhibited, the crossed extensor reflex was easily obtained. In another experiment, after obtaining the reflex responses and their inhibition by vibration, the animal was subjected to a bilateral lumbosacral sympathectomy. This procedure had no observable effect on either the patellar reflex or its inhibition by vibration. Subsequent stripping of the adventitia from about a centimeter of femoral artery just below the fossa ovalis left both the reflex and its inhibition unchanged. In another cat a unilateral sympathectomy was performed and both thighs examined as described above. No difference in either the reflex or its inhibition was found between the two sides.

It is evident from the above findings that the inhibitory action of vibration need have no direct dependence on the presence of sympathetic pathways. The likelihood is, therefore, that the inhibition is only apparent. The vibration itself may stimulate the reflex periodically and thus make it very difficult for a superimposed stimulus to be effective. The tapping of the tendon is apparently a stimulus of too great duration to be effective in competition with vibratory stimulation at 100 cycles per second. However, stimulation at frequencies low enough to give a period longer than the duration of a tap should permit at least occasional tapping stimuli to produce a reflex. In order to test this, the vibrator was applied at frequencies of 10, 15, 20, and 30 cycles. It was then observed that with 10-cycle vibration most of the tendon taps produced a response. At 15 cycles only about one half of the taps produced a response. At 20 cycles, roughly one-quarter were effective, and at 30 it was only rarely possible to obtain the reflex contraction by tapping.

CARBAMATE CONDUCTION BLOCK IN FROG NERVE FIBERS

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I N A previous communication (1) evidence was given for the appearance of a small, but significant increment in resting potential in a region of living frog sciatic nerve treated with any one of a series of four homologous carbamates. In the concentrations which elicited this positivity the carbamates also produced a readily reversible conduction block in the *A*, *B* and *C* fibers. It is the object of this report to describe certain features in the action of these narcotics in producing this conduction block.

EXPERIMENTAL PROCEDURE

The procedure has already been described in some detail (1). The carbamates (ethyl-, n-propyl-, n-butyl- and n-amyl-carbamate) were dissolved in phosphate-Ringer's solution at a *p*H of 7.3 and applied to a 25 mm. segment (*segment A*) of isolated frog sciatic nerve. The central sciatic stump was stimulated at a rate of 6 to 12 shocks per minute by means of an electronic stimulator. The monophasic action potentials, recorded oscillographically, were led off from the nerve distal to *segment A*. Conduction block in *segment A* was therefore indicated by a gradual decrease in the spike height. The shock strength and sweep were adjusted differently in different experiments so as to study the impulses in the *A* fibers alone, the *A* and *B* fibers or the *B* and *C* fibers. The experiments were performed at the constant temperature of 25°C. All other conditions were as previously described (1). In this investigation 41 nerves were employed. Of these, 38 were from the bullfrog (*Rana catesbiana*) and three from the grass frog (*Rana pipiens*).

RESULTS

Effect of Previous Treatment

When any one of the carbamates was added to *segment A* in place of Ringer's solution there occurred a gradual reduction in height of the *A* (fig. 6), the *B* (fig. 3 and 5) and the *C* spikes (fig. 5). A plot of the spike amplitude (in percentage of the original height) against time gave curves (figs. 1, 2 and 4) which describe the course of block well enough for purposes of this report. Such curves permitted the selection of some arbitrary point, e.g. the time for reduction of the spike to the 50 per cent level, which then served as a descriptive time characteristic for the block action. When a nerve was tested repeatedly during the course of 15 to 20 hours with the same concentration of any one of the carbamates the time course of block for any one group of fibers varied from test to test. One important factor in this variability was

TABLE 1. THREE-TEST EXPERIMENT ON 12 NERVES

A NERVE	B TEST	C DRUG	D MOLARTIY	E TIME OF TEST, MIN.	F MINUTES TO 50% A HEIGHT	G ½	H ½
11	1	E	0.210		14.0		
	2			15	5.0	2.80	
	3			80	12.0		1.17
13	1	P	0.070		10.2		
	2			15	8.3	1.23	
	3			85	11.2		0.91
15	1	A	0.007		6.0		
	2			15	4.4	1.37	
	3			70	9.6		0.63
16	1	B	0.025		9.0		
	2			15	7.4	1.22	
	3			80	9.0		1.00
17	1	A	0.008		14.8		
	2			15	8.8	1.68	
	3			57	13.2		1.12
18	1	B	0.020		15.1		
	2			15	10.8	1.40	
	3			57	13.2		1.14
19	1	P	0.080		15.0		
	2			15	10.3	1.46	
	3			60	17.4		0.86
20	1	E	0.210		19.8		
	2			15	12.7	1.56	
	3			70	23.0		0.86
68	1	A	0.007		30.0		
	2			20	11.6	2.59	
	3			60	32.3		0.93
70	1	A	0.007		13.6		
	2			15	8.8	1.55	
	3			60	17.2		0.79
71	1	P	0.060		8.4		
	2			6	7.3	1.15	
	3			60	11.3		0.74
72	1	P	0.070		8.4		
	2			8	5.4	1.56	
	3			72	7.1		1.18
Means...						1.63	0.04

The carbamates are indicated as E (ethyl), P (n-propyl), B (n-butyl), A (n-amyl). In *column E* are indicated the times after washing out the previous carbamate that the indicated tests were made. The ratios of the times to 50 per cent reduction of the *A* spike of the test 1 to test 2 (*column G*) and the test 1 to test 3 (*column H*) figures are given.

the occurrence of a residual sensitivity in the nerve which remained after exposure to carbamate. A typical illustration of this effect for the A group of fibers is given in figure 1A. Curve 1 shows the block action following the initial treatment with carbamate; in this case amyl carbamate, 0.008M. Recovery of the A spike required about 6 minutes after removing the carbamate and washing out *segment A* with Ringer's solution (fig. 1B). A second and similar test was then repeated on the same nerve at 15 minutes after removal of the carbamate of the first experiment. Block (curve 2) developed earlier, although recovery was much the same as with the first experiment. A third repetition of the experiment (curve 3) at 57 minutes after removal of the carbamate of the second test demonstrated that the time course of block was approximately similar to that of the initial experiment. A summary of the data (table 1) on 12 nerves offers statistical evidence that the course of block in the A fibers was significantly faster when the nerves were tested 15 minutes or less after removal of the carbamate of the first test than if the nerves were tested an hour or
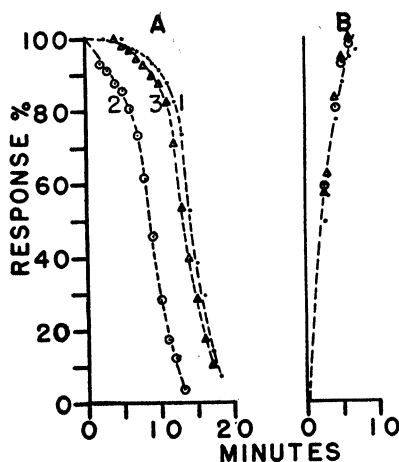
Fig. 1. TIME COURSE of block (A) and recovery (B) of the A fibers following the addition and removal of amyl carbamate (0.008M). Data for curve 1 (*points*) were obtained after the initial addition of drug. Curve 2 (*circles*) shows the results of the second addition of drug at the 15-min. point in the recovery from *exper. 1*. Curve 3 (*triangles* was obtained at the 57-min. recovery point of *exper. 2*.

longer after the removal of the drug of the second test. Thus in a comparison of the ratios for the 50 per cent block time (table 1), the algebraic mean of the test 1 to test 2 ratio was 1.63, significantly greater than unity, whereas the corresponding mean for the test 1 to test 3 ratio was 0.94, not significantly different from unity. The occurrence of a heightened sensitivity which persisted for a time after removal of the carbamate and then disappeared may be related to the residual positivity which was previously shown (1) to outlast the conduction block. It is not known whether these residual effects represent true delays in recovery of those physiological processes which were affected by the carbamate molecule or whether they represent the action of residual carbamate which was only slowly removed after washing.

Most of the experiments to demonstrate the residual sensitivity were carried out with the A spikes as indices of response. A few tests made on the B fibers indicated that the same phenomenon also appeared with this group. No examination was made of the C fibers for residual sensitivity.

Relative Activity of the Different Carbamates

The alkyl carbamates are known to be surface active compounds whose surface activity increases as the alkyl chain is lengthened.   If conduction block involved an adsorption of the narcotic at a surface, the effectiveness of these compounds in producing block should increase in going from the ethyl to the amyl ester.   This increase was actually observed.   The time course of block of the A fibers with different concentrations of any one carbamate varied in the manner indicated by the curves of figure 2A, obtained with n-butyl carbamate.   From these and similar curves it was
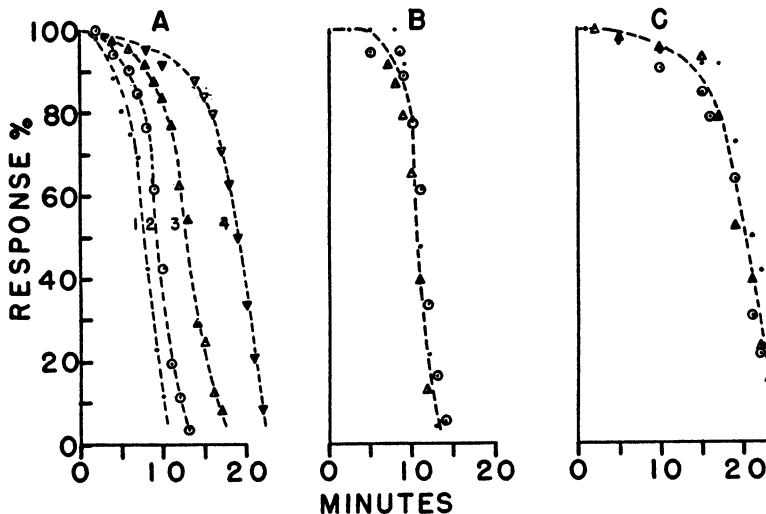
Fig. 2. SERIES A.   Time course of block in the A fibers with butyl carbamate of concentrations 0.025M(1), 0.023M(2), 0.020M(3) and 0.018M(4).   At least 1 hr. was allowed for recovery between each experiment.   SERIES B.   Approximately matched responses of a nerve to 3 carbamates: propyl carbamate (0.07M), shown as *points;* butyl carbamate (0.018M), as *circles;* and amyl carbamate (0.0065M), as *triangles.*   Liquid-air surface tensions of these 3 solutions, as interpolated from curves of surface tension versus concentration (measurements with a tensiometer) were 61.7, 62.8 and 57.5 dynes/cm., respectively.   SERIES C.   Approximately matched responses of a nerve with: ethyl carbamate (0.21M), as *triangles;* propyl carbamate (0.07M), as *points;* and butyl carbamate (0.018M), as *circles.*   The liquid-air surface tensions of these 3 solutions were 62.8, 61.7 and 62.8 dynes/cm., respectively.

then possible to compare the concentrations of the different carbamates which gave approximately the same block curve.   The relative effectiveness of the propyl, butyl and amyl esters are compared in figure 2B while a similar comparison for the ethyl, propyl and butyl compounds is shown in figure 2C.   The carbamate concentrations which gave these approximately matched pharmacological responses also reduced the surface tension of Ringer's solution to about the same degree (fig. 2 legend).   The relative effectiveness of these compounds in blocking conduction was thus roughly of the same order as their ability to reduce surface tension.

Differential Sensitivity of the Fiber Groups   •

A. *B fibers.*   Much evidence was obtained to show that the different fiber groups of the bullfrog sciatic-peroneal nerve responded quite differently to these narcotics.

In this action all four carbamates behaved similarly. The most sensitive fibers proved to be those of the *B* group. These ceased conducting before either the *A* or the *C* fibers. A typical example of the differentiation between the *B* and *A* groups is shown in figure 3. In a number of experiments the *B* spike was completely abolished while the *A* spike was still at its original level, indicating that all the *B* fibers were blocked before any significant number of *A* fibers had ceased conducting (fig. 4). In recovery, the *B* fibers, as a group, lagged significantly behind the *A* fibers (fig. 4). The data from 11 experiments show that on the average the time to reduce the spike to half its original value is about three times as great for the *A* as for the *B* group of fibers (table 2).
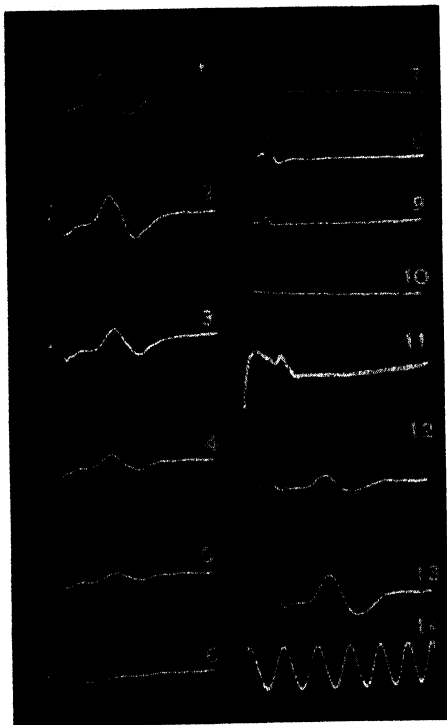
Fig. 3. DIFFERENTIAL CONDUCTION BLOCK of the *A* and *B* fibers by propyl carbamate (0.07M). Record 1 is the Ringer's sol. control. The others were photographed 4′(2), 7′(3), 8′(4), 9′(5), 10′(6), 13′(7), 18′(8), 24′(9) and 30′(10) after adding the drug. Recovery is shown at 4′(12) and 7′(13) after washing out the drug. The 60 c.p.s. time line is in record 14. Record 11 indicates that some of the *C* fibers were still conducting after both *A* and *B* groups were all blocked.

A comparison of the *B* and *C* fibers is made in figure 5. In this experiment complete block in the *B* fibers occurred with no significant reduction in the C_2 action. Two experiments in which the *C* spike was sufficiently developed to permit accurate measurements are included in the data of table 2.

B. *A fibers.* As is well known (2) the *A* fibers of the bullfrog sciatic nerve consist of several groups which appear as discrete peaks in the compound *A* spike. It is of interest to inquire whether these components of the *A* group can be differentiated in any way through the action of the carbamate molecule. Generalization is made difficult and uncertain because of the varying behavior of different nerves or of even the same nerve at different examinations. Ignoring these variations, the results as a whole indicate some differentiation in the *A* fibers. The most prominent feature

of this differentiation was that the smallest (gamma) fibers were most resistant. A typical experiment (fig. 6) illustrates the persistence of some gamma fibers after all the alpha and beta fibers were blocked. A similar differentiation was obtained in eight nerves in which the gamma spike was distinct enough to be recognizable.
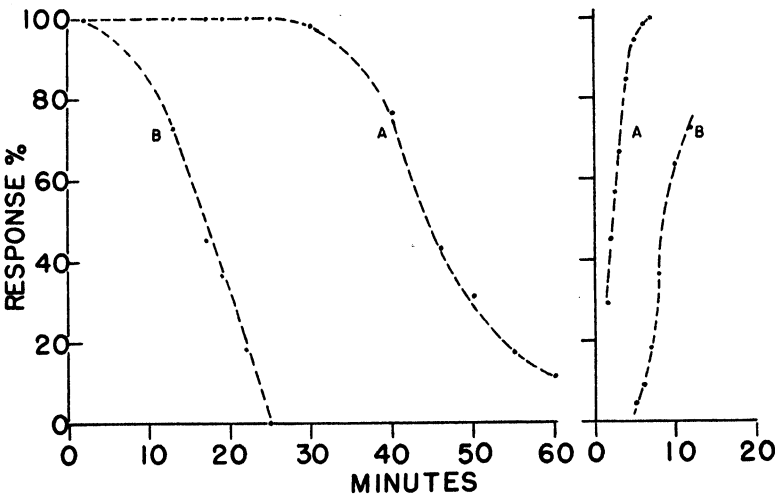
Fig. 4. TIME COURSE of block and recovery in A and B fibers following the addition and removal of propyl carbamate (0.07M).

TABLE 2. DIFFERENTIAL SENSITIVITY OF FROG NERVE FIBERS

NERVE	DRUG	MOLARITY	MINUTES TO 50% BLOCK			RATIO OF A TO B BLOCK TIME
			A	B	C	
26	P	0.06	66.0	18.5		3.6
27	B	0.02	25.0	6.0		4.2
28	P	0.08	19.2	10.0		1.9
28	A	0.007	29.5	9.0		3.3
29	B	0.023	18.4	9.0		2.0
31	P	0.10	8.2	5.0		1.6
35	A	0.008		4.6	15.5	
35	A	0.008	14.3	6.5		2.2
68	A	0.007	30.0	4.1		7.3
68	A	0.007	32.3	7.1		4.5
73	P	0.07		9.3	52.7	
73	P	0.07	12.8	7.9		1.6
76	P	0.07	45.0	16.9		2.7
Mean..........						3.2

C. *C fibers.* It has already been indicated that the C fibers as a group blocked less rapidly than did the B fibers. A simultaneous comparison of the C and A fibers was not made because of the large artifact introduced into the A spike as a result of the increased shock strength and increased amplification required to bring out the C spikes. It was possible to show, however, in a number of experiments that when
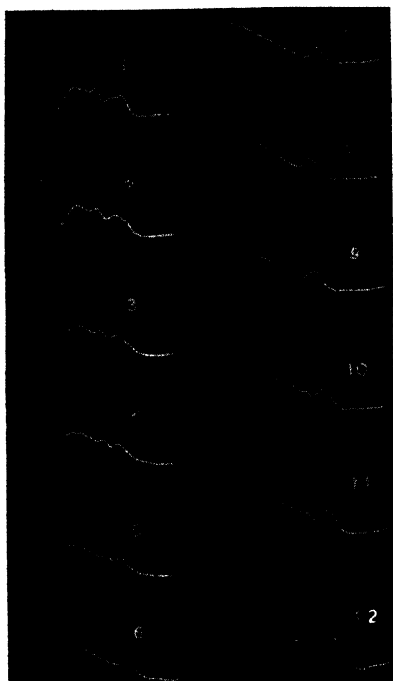
Fig. 5. DIFFERENTIAL CONDUCTION BLOCK in the B and C groups of fibers by propyl carbamate (0.07M). Record 1 is the Ringer's sol. control. The drug action is shown at 2'(2), 4'(3), 6'(4), 8'(5), 12'(6), and 45'(7). Recovery is indicated at 1.5'(8), 2'(9), 3'(10), 6'(11) and 10'(12). Note persistence of the C_2 action long after both B and C_1 potentials had been abolished.
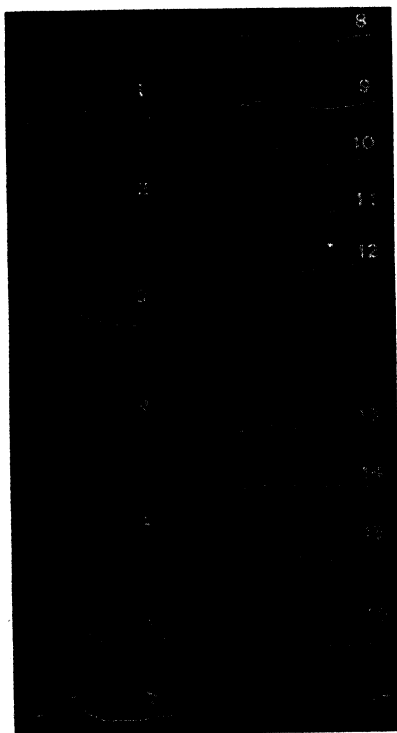
Fig. 6. CONDUCTION BLOCK in the A group of fibers. Record 1 shows the A spikes with segment A in Ringer's sol. The differential block by butyl carbamate (0.017M) is indicated in records 2–12, taken 14'(2), 20'(3), 30'(4), 34'(5), 38'(6), 43'(7), 48'(8), 51'(9), 56'(10), 68'(11) and 72'(12) after adding the drug. Amplitudes are not directly comparable in all records as the amplifier gain was increased between records 3 and 4 and decreased again between records 12 and 13, indicated by change in size of the shock artifact. Recovery, incompletely indicated by records 13–16, occurred after washing out segment A. These records were made at 1.3'(13), 2.3'(14), 5'(15), and 8'(16) after washing. Record 17 is of the 1000 c.p.s. time line.

both B and A fibers had ceased conducting, an appreciable proportion of the C spike was still present. This is illustrated by the experiment in figure 3. Records 2 to 10 show the disappearance of first the B, then the A spike, so that record 10 shows no evidence of either A or B fiber activity. Record 11 was photographed one minute after record 10. It pictures the C action resulting from the increase in shock strength. The C spike in this record was about 60 per cent of the original C height at the beginning of this series. This experiment demonstrates the relative insensitivity to carbamate of some C fibers.

The C spike of the bullfrog sciatic-peroneal nerve was composed typically of two components. In these experiments indications were obtained that C_1 and C_2 were also capable of being differentiated by the carbamates (fig. 5). The evidence at hand on this point is neither abundant enough nor certain enough to permit any further statements at this time.

DISCUSSION

Essentially, this report describes certain phenomena observed during conduction block in frog nerves treated with relatively dilute concentrations of the carbamates. Neither here nor in the existing literature is there sufficient information to permit a conclusion regarding the mechanism of action of these compounds in producing conduction block. Nevertheless, certain statements may be made which assist in integrating some of the published data. In a previous report (1) reasons were presented for the concept that the increase in resting potential following treatment of the nerve with these compounds may be associated with a decrease in permeability. Evidence already exists suggesting that carbamates in the proper concentrations are able to decrease the permeability of a number of cells (3–6). There is good agreement between the figures for the concentrations which have been reported to decrease permeability (5, 6) and which here have been shown to produce a readily reversible conduction block.

At present a student of the nerve impulse is presented with two working hypotheses: 1) that conduction is associated with a transient increase in permeability (7, 8) and 2) that conduction block, at least with the carbamates, is associated with a decrease in permeability. It is of course a key problem and one which is the cause of much controversy to decide whether the passage or release of some specific substance is the primary event in conduction and if so to reveal the nature of this substance. Narcotics, such as the carbamates, might then be visualized as causing block by interfering with the release of or preventing the passage of such an essential substance. The immediate problem appears to be whether substances like the carbamates may interfere with the release or transfer of K^+. Hodgkin and Huxley (8) recently reported that the increased conductance following activity in isolated axons of *Carcinus maenas* was associated with the release of a substance having effects on conductance similar to the effects of K^+. Shanes (9) has also considered this problem in relation to the mode of action of cocaine. He concluded that the permeability of muscle and nerve to K^+ is reduced by this narcotic. All these studies open several paths for future investigation.

The differential fiber sensitivity of frog nerves to carbamates resembles the

differential effects of hypoxia or anoxia. It has been shown (10, 11) that the *B* fibers in mammalian nerves are most quickly blocked by lack of oxygen while the *C* fibers are the most resistant. A similar behavior of bullfrog nerves has recently been reported (12). The similarity in response of nerve to anoxia and to the carbamates may be simply coincidental, but it is of interest to point out that the carbamates in the same range of concentrations as employed in this work are able to depress the oxygen consumption of many living cells (13–16).

The differential blocking action of the carbamates does not resemble the action of other narcotics. Cocaine, for example, has been reported to block the *C* fiber group before the *A* fibers (17). Procaine appears to have a similar action (18). Moreover, in contrast to the carbamate type block, cocaine is known to affect frog and mammalian *A* fibers differentially, with the smaller fibers blocking first (17, 19). Ether and nembutal have been reported (20) to act differentially on the fibers of the turtle vagus nerve with the *C*, *B* and *A* fibers blocking in the order named. In an interpretation of the selective action of cocaine on the *A* fibers, Gasser and Erlanger (19) suggested that either the thinner myelin sheath or the greater surface per volume ratio might be responsible for the greater susceptibility of the smaller fibers. It is obvious that neither of these suggestions is adequate to explain the carbamate type of differentiation. Lorente de Nó (12) has also emphasized the point that the thickness of the myelin sheath appears to have little to do with the action of a number of substances. The work of the Japanese investigators (21) showing that the nodes of Ranvier are regions of particular sensitivity to narcotics may render irrelevant any consideration of the myelin sheath as a barrier to the penetration of substances.

SUMMARY

Certain features in the blocking action of ethyl-, n-propyl-, n-butyl- and n-amyl carbamate are described. These esters were able to block selectively the different fibers of the bullfrog sciatic nerve. The *B* fibers as a group were most readily affected. The time to reduce the spike height to 50 per cent of its original level was about three times as great for the *A* fibers as for the *B* fibers. Among the *A* fibers, the gamma fibers were typically most resistant. There was some difference in behavior of the different *C* fibers but a large proportion of them were able to conduct after all *A* and *B* fibers had been blocked. The relative effectiveness of the four different carbamates in blocking the *A* fibers was found to be approximately the same as their relative effectiveness in reducing surface tension. Evidence for the existence of a period of heightened susceptibility to block by carbamate was noted following the washing out of a previously added carbamate and the complete recovery of ability to conduct.

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SHOCK DUE TO HEAD INJURY IN THE FROG

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D EATH from head injury in the absence of demonstrable histo-pathological changes has long been an enigma (1-3). Evidence has been reported (3) for the rôle of a reversible 'molecular reaction' in the concussional state. In this report the concussional state was shown to be independent of vascular spasm, hemorrhages, stasis of the brain, fat embolism or any other detectable histopathological lesions. Earlier work in this laboratory (4) has shown that in electrical injury to the brain there is released into the blood stream a toxic factor that has thromboplastic properties. There are other suggestions of a possible connection between the phenomenon of toxicity and thromboplastic properties. The placental toxin, which is considered to play an etiological rôle in eclampsia, has been shown by Schneider to be thromboplastin (5). Moreover, thromboplastin has been shown to be involved in producing some of the toxic effects of muscle ischemia (6). Most of the literature on the relation of injury to protoplasmic clotting has been reviewed already by Heilbrunn (7-9).

The question arises as to whether head injury could be followed by the release of thromboplastic substances into the blood. If this is true, head injury should be followed by disturbances of the blood coagulation. The brain is an especially rich source of thromboplastic material (10, 11), and brain extracts have been shown to be highly toxic (12, 13). Thus, it is possible that head injury can produce toxic effects not immediately due to nerve impulses. Accordingly, it was attempted to determine whether visible toxic effects would follow head injury in animals whose spinal cords had been severed; and, also, to record the associated changes in the blood-clotting time.

METHODS

Frogs, *Rana pipiens*, weighing 18 to 23 grams and of both sexes were chosen as the experimental animal. In all, 422 animals (including 164 controls) were used. Earlier experiments were performed without any special equipment. Head trauma was produced by grasping the frog and striking its head on the edge of a table. Afterwards, trauma was given with a light hammer, a stiff rubber tube or by crushing the head with narrow pliers. The best results were obtained with a traumatizing device. This device consisted of an arm powered by a rachet wheel so that it could be brought down repeatedly on the head of the animal with uniform force. Each turn of the wheel caused the arm to fall four times.

In order to eliminate the effect of nerve impulses and muscular fatigue attendant

upon the convulsive effects of head trauma, the spinal cord was severed by means of a small knife made of flattened iron wire approximately 2 mm. wide. A small transverse incision was made over the foramen magnum with a scalpel. Then the knife was inserted straight across the spinal cord and moved sidewise two or three times. The main difficulty with this procedure lies in the excessive bleeding that may occur upon the accidental rupture of the spinal vessels. All animals that showed excessive bleeding were eliminated from the experiments, for the reason that excessive bleeding leads to hemodilution, which results in prolongation of the clotting time.

The clotting time of the blood was determined by the capillary method. Samples were obtained from the exposed heart, by puncture with a capillary tube. The details and precautions of this method are given in the earlier paper (4).

<center>EXPERIMENTS</center>

General Effects of Head Injury upon the Frog with Transected Spinal Cord. In preliminary experiments 55 frogs were given head trauma by relatively inexact methods: striking on the table, using the light hammer, striking with a stiff rubber tube and crushing with pliers. Of these 55 animals, 14 went into irreversible depression in 30 to 60 minutes; 14, in 6 to 8 hours; 10, in 8 to 10 hours and 12, in 12 to 24 hours.

It was noted that most of the animals with the longer survival time had received greater apparent injury: the skull had been crushed by the trarᵥnatic procedure. Most of the animals with shorter survival times had received less apparent injury. From these results it appeared that if a toxic factor was produced it did not always gain access to the general circulation. Occlusion of blood vessels resulting from the excessive bone and tissue crushing was thought to be involved.

The traumatizing machine was then made for the purpose of miminizing the crushing effects of trauma. With the arm adjusted so that the force of each tap was approximately 500 grams, 10 frogs were traumatized with 200 taps. When this method is properly administered in frogs (after transection of the spinal cord) it is not attended by any noticeable convulsive effects. The hind legs remain in a natural position. After the trauma there is a brief period (approximately 30–45 seconds) in which the hind legs are not responsive to pricking with a needle; but after this, they become normally responsive, although the frog does not move about on its own initiative. None of the 10 frogs traumatized with the 500 gram force was seen to show any signs of depression for at least 8 hours.

The arm was readjusted so that the force of each tap would be approximately 725 grams. Of 12 frogs given 200 taps, 10 were in irreversible depression within 35 minutes. The remaining 2 succumbed in 4 hours. In frogs weighing 18 grams or more, this method of traumatizing produces no bone-crushing effects. This method was adopted for all the remaining experiments.

The observable characteristics of depression due to head trauma were as follows: for about 30 to 45 seconds following trauma the hind legs were flaccid and unresponsive to prodding. After this they recovered normal reactivity for a variable period, but most commonly for 30 to 60 minutes; then a generalized depression followed.

This depression was characterized by a gradually decreasing responsiveness of the front and hind legs, accompanied by sluggishness of the corneal reflex. The corneal reflex sometimes outlived the responsiveness of the legs by 15 to 20 minutes, but sometimes was entirely lost before the legs became altogether unresponsive to mechanical stimuli. Usually about 15 to 20 minutes were required for the complete loss of reactivity of the legs. The heartbeat continued with gradually decreasing strength, force and rate for $2\frac{1}{2}$ to 4 hours after the onset of depression.

Depressant Effect of Transection of Spinal Cord. Spinal transection itself, without trauma to the head, produces signs of irreversible depression after a few hours. Seventeen frogs were transected and placed in a pan containing a small amount of water. The survival time of these animals ranged from 1 to 20 hours (av. = 4.3 hours).

Another experiment was done in which 20 frogs were transected, but 10 of these were given head trauma, in addition. The animals were placed in similar containers and observed. The results are shown in table 1, which shows that head trauma shortened the average survival time by 75 per cent.

TABLE 1. COMPARISON OF THE SURVIVAL TIME OF FROGS AFTER TRANSECTION OF THE SPINAL CORD WITH AND WITHOUT HEAD TRAUMA

NO.	1	2	3	4	5	6	7	8	9	10	AVER- AGE
Survival time; (hr:min.)											
Transection and trauma	0:05	0:10	0:30	0:30	0:30	0:25	1:20	1:40	2:30	2:50	1:03
Transection	0:45	1:40	2:40	3:20	3:20	5:00	5:30	6:00	6:00	7:30	4:04

Effects of Head Trauma on Blood Coagulability. A preliminary study made by Mr. J. T. Hicks, in this laboratory, showed that clotting time increased after head injury. The average clotting time in 128 untraumatized frogs was 3.6 minutes; in a separate group of 128 frogs, the clotting time 20 minutes after trauma was found to be 12.1 minutes. In these experiments the spinal cord was not transected. Trauma was given by striking the frogs on the edge of a table. The results of this experiment differ somewhat from those of other experiments and will be commented on later.

Using the traumatizing machine after cord transection, the clotting time was found to be decreased immediately after trauma. Sixteen animals with transected cord were used as controls, and had an average clotting time of 3.2 minutes. In 10 animals with both cord transection and head trauma the average clotting time, immediately after trauma, was 1.0 minute.

Another experiment on 5 frogs in which the clotting time was determined in each frog immediately before and after trauma also indicated a decrease, though not as marked, as in the previous experiment. The average clotting time was one minute, 47 seconds before trauma, and one minute, 7 seconds after trauma.

The next experiment consisted of serial determinations in which, for each animal, the clotting time was determined at intervals over a period of time. Eleven test animals and 11 controls were used. The test animals fell into two almost equally divided groups: one with a relatively high initial clotting time and the other with a relatively low initial clotting time. This difference in clotting times was
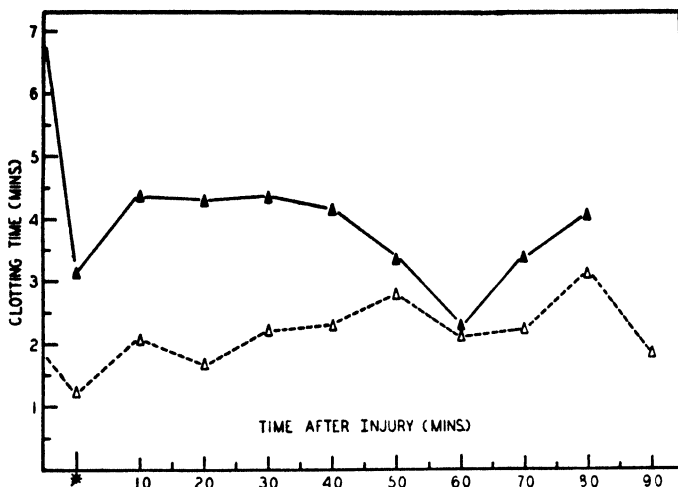
Fig. 1. EFFECT OF HEAD INJURY ON BLOOD COAGULABILITY OF FROG. *Upper curve,* average values obtained for 6 frogs. It was not possible to obtain blood samples from one of the animals after 40 min., and from 2 other animals after 50 min. After these points the curve represents values obtained from the 3 remaining animals. *Lower curve,* averages obtained from 5 animals. Sampling became impossible for 2 of the animals after 50 min.
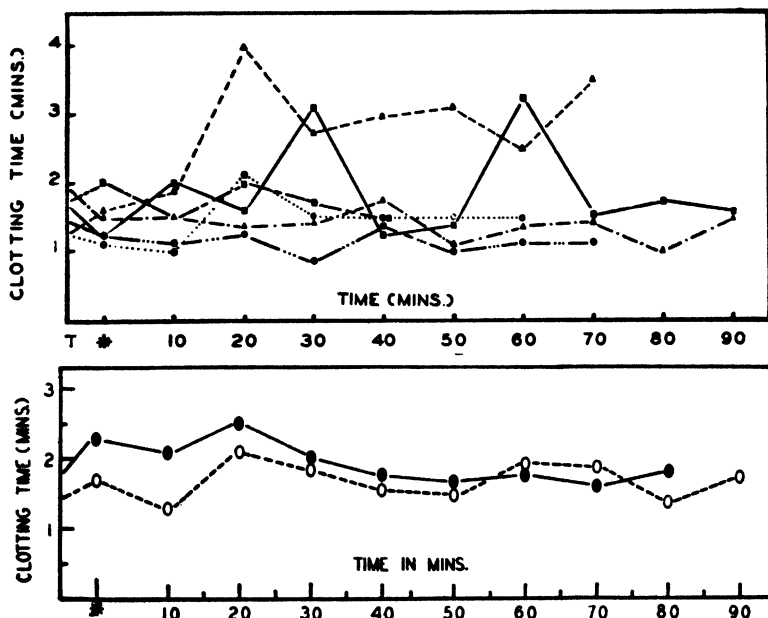
Fig. 2. CONTROLS (*upper*). Changes in blood-clotting time of 6 frogs with transected spinal cord, but no head injury.

Fig. 3. CONTROLS (*lower*). Changes in blood coagulability in frogs which were not given head injury. *Upper curve* represents those with transection of the spinal cord; *lower curve* represents those which were given no treatment.

encountered in frogs used in studying the effects of electrical injury (4). Possibly the reason for the differences in clotting time in these animals is that the experiments

were done during the summer. The frogs had been transported during the hot weather and some of them might have suffered some degree of heat injury. Nevertheless, whatever the cause of the difference in clotting time it is not considered significant from the point of view of these experiments, since the effects of trauma were substantially similar in both the high and the low groups (fig. 1). In these experiments the clotting time 20 minutes after trauma was not elevated. This differs greatly with the results of the previously mentioned experiment in which the spinal cords of the animals were intact and only one blood sample was taken. In this experiment the clotting time was distinctly elevated at 20 minutes after trauma. However, no definite statement can be made at this time to explain the difference.

Eleven frogs served as controls. In 6 of the controls spinal cord transection was done before determining the clotting times. The results were similar in both groups. An idea of the degree of individual variation can be obtained from figure 2 which shows the curves obtained from the control animals with cord transected. Figure 3 shows the average values obtained from the controls.

DISCUSSION

The foregoing experiments have shown that mechanical injury of the head can produce delayed, irreversible depression in the frog. The experiments were planned so as to eliminate factors other than circulatory. No amount of head injury was able to cause death when the injury was such as to crush the brain case and thus to impede the exit of cerebral blood to the general circulation. Therefore, it is most reasonable to deduce that the depression observed was due to the release of some toxic factor from the injured head into the bloodstream.

In the past it has been abundantly shown that both anaphylactic shock and clinical shock are accompanied by prolongation of the blood-clotting time (14, 15). Generally, it has been held that the prolongation of the clotting time, due to an excess of blood heparin, is the manifestation of a mechanism counteracting some increased thrombogenic tendency of the blood. In studies of tourniquet shock by Mylon *et al.* (16) release of the tourniquet was followed by an immediate decrease in the blood-clotting time. This was followed by an increase in clotting time. In the present experiments, there was obtained evidence for the rôle of a thromboplastic substance in shock due to head injury. It was observed that injury was followed by a disturbance of blood coagulation. This disturbance is characterized by an initial decrease in clotting time, followed by greater or lesser increases and decreases.

The present experiments are corroborative of earlier experiments (to which reference has already been made, 4) on the effects of electrical injury. In these earlier experiments shock was accompanied by disturbances of blood coagulation similar to those in head injury. Moreover, in the experiments on electrical injury of the hind legs, the depth of depression could be controlled by the alternate application and removal of ligatures proximal to the injured areas.

The findings recorded in these experiments are in accord with the theory that in injury shock a circulating toxic factor is involved; and that this toxic factor is a substance with thromboplastic properties.

SUMMARY

Mechanical injury of the head produced profound physiological depression and death in frogs whose spinal cords had been transected. Regardless of the amount of trauma, the toxic effects were absent or decreased when trauma was accompanied by obstruction of the cerebral blood flow due to crushing of the skull. These observations suggest the presence of a circulating toxic factor. Disturbances in the blood-clotting mechanism after injury indicate that the toxic factor is a substance with thromboplastic properties. These results are correlated with the results of previous work favoring the concept that thromboplastic substances may be involved in the pathogenesis of injury shock.

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PULMONARY CAPILLARY PRESSURE IN ANIMALS ESTIMATED BY VENOUS AND ARTERIAL CATHETERIZATION

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B
Y MEANS of the technique of venous catheterization of Cournand and Ranges (1), it has been possible to record pressures in the great veins, right auricle, right ventricle and pulmonary artery with suitable recording devices. This study describes a method by which the pressure in the pulmonary capillaries can be estimated by means of the venous catheter.

METHODS

Dogs anesthetized with nembutal (40 mg/kg. intraperitoneally) were used in the present study. A no. 8 French catheter, 2.5 mm. in diameter, with a hole in the tip, was introduced into the external jugular vein and guided fluoroscopically, as described elsewhere (2), into a branch of the pulmonary artery. It was pushed as far as possible and wedged in a distal ramification so as to obstruct the vessel.

A no. 8 French catheter was introduced by way of the carotid artery into a pulmonary vein on the same side of the lung as the first catheter (3). The carotid artery was exposed and two loops of silk served to lift the artery out of the wound and to occlude blood flow temporarily. A small opening was made through the arterial wall with a sharp no. 14 needle and a no. 8 French catheter was introduced through this hole into the arterial lumen. If a snug fit was obtained, no bleeding occurred and the silk stays could be removed. The catheter was guided fluoroscopically into the ascending aorta by keeping the curved tip to the animal's right. When resistance was encountered at the aortic valve the catheter was introduced through the valve into the left ventricle by gently withdrawing and advancing the catheter until it slipped through the valve during ventricular systole. The tip was then directed posteriorly and to the right and inserted further causing a bend to appear with its convexity to the left and with the tip pointing medially and superiorly in the direction of the mitral valve. It was then advanced through the valve into the left auricle and into one of the pulmonary veins. Here it was wedged so as to obstruct the lumen of the pulmonary vein.

A third catheter, no. 9 French, was introduced through the other jugular vein into a branch of the pulmonary artery of the opposite lung, but was not allowed to obstruct the vessel. This catheter was used for recording pressure in the pulmonary artery and for the injection of embolic material. Pulmonary embolism was produced by injecting through this catheter a one per cent suspension of lycopodium spores at the rate of 3 cc/min. into a local area of the lung distant from the location of the other two catheters. The purpose of this was to raise the pressure in the pulmonary artery, pulmonary capillaries and pulmonary vein (4). Figure 1 is an X-ray of the chest with the three catheters in position. To determine the effect of respiration on the pressures recorded in the ob-

structed artery and vein, two experiments were performed with controlled respiration after the spinal cord had been pithed.

The position of the catheters was verified by chest X-rays taken during the experiment, and all dogs were autopsied to confirm the location of the catheters and to allow pathological inspection of the cardiac chambers and lungs.

Pressures were recorded with both the optical manometer of Hamilton (5) and a column of saline as is commonly used for measuring venous pressure. The zero point for all pressures was taken 7.5 cm. anterior to the spine with the dog lying on its back.

RESULTS

The contours of the pressure curves recorded through the catheters occluding the pulmonary artery and vein were similar. As can be seen in figure 2, no pulse wave

Fig. 1. ANTERO-POSTERIOR ROENT-GENOGRAM of chest showing location of catheters. *a.* Obstructing right lower lobar branch of pulmonary vein. *b.* Obstructing right lower lobar branch of pulmonary artery. *c.* Free in left lower lobar branch of pulmonary artery.

was obtained but there was considerable respiratory variation in the recorded pressure, the pressure being lower in inspiration than in expiration by an average of 7 mm. of Hg (tables 1 and 2). The small waves in the tracing were artifacts, presumably resulting from the bending of the catheter during ventricular systole.

Mean pressures recorded through the catheter wedged into the pulmonary artery in 24 dogs varied between 2 and 8 mm. Hg with an arithmetical mean of 4 mm. Hg (tables 1 and 2). In 6 dogs in which the mean pressure was recorded simultaneously through the catheter obstructing the pulmonary vein the pressure was uniformly higher, on repeated determinations in each experiment, by an average of 4 mm. of Hg (table 1). This same relationship was maintained in both inspiration and expiration with the exception of *dog 82* in which it was reversed in both phases of respiration. However, in this animal the mean pressure through the catheter obstructing the pul-
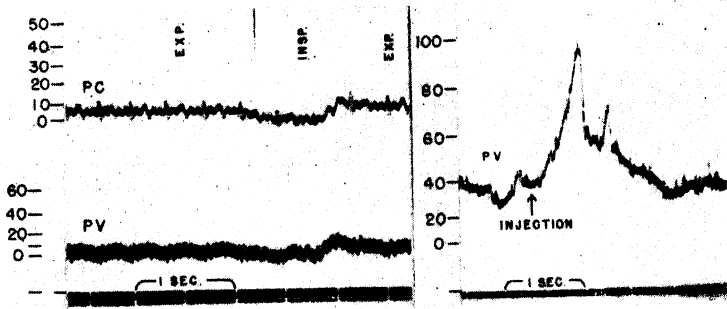
Fig. 2 (*left*). SIMULTANEOUS RECORDING, with Hamilton manometer, of pressure in blocked pulmonary artery (P.C.) and blocked pulmonary vein (P.V.). Note that contours of curves are identical.

Fig. 3 (*right*). RECORDING OF PRESSURE in obstructed pulmonary vein (P.V.) showing rapid increase in pressure resulting from injection of 1 cc. of saline through the opposing catheter wedged into the pulmonary artery.

TABLE I. VARIATIONS IN PRESSURE IN AN OBSTRUCTED PULMONARY ARTERY AND VEIN IN 6 NORMAL DOGS AND IN 4 DOGS WITH PULMONARY HYPERTENSION AS A RESULT OF PULMONARY EMBOLISM

All values in mm. Hg

EXP. No.	PRESSURES WITH CATHETER IN										EST. PULMONARY CAPILLARY PRESSURE WITH FREE FLOW	
	Pulmonary artery		Pulmonary end artery				Pumonary end vein					
	Hamilton manometer		Hamilton manometer			Saline manometer mean	Hamilton manometer			Saline manometer mean	Hamilton manometer mean	Saline manometer mean
	Syst.	Diast.	In-spir.	Ex-pir.	Mean		In-spir.	Ex-pir.	Mean			
67	45	20	5	12	8	5	8	13	10	12	0	8.5
68	26	10	1	10	6	6	11	18	14	14	10	10
70	26	18	2	10	5	6	6	11	9	9	7	7.5
73	26	9	4	12	7		10	14	12	16	9.5	
82			4	9	5	8	2	8	6	9	5.5	8.5
83			2	8	6	6	6	15	11	11	8.5	8.5
Average....	31	14	3	10	6	6	6	13	10	12	8.3	8.6

After onset of pulmonary hypertension produced by injection of lycopodium spores into pulmonary artery of opposite lung

67	80	48	4	14	10	14	8	17	15	22	12.5	18
68	64	35	8	18	13	11	14	30	22	27	17.5	19
70	88	38	11	18	14	16	14	21	19	20	16.5	18
82	70	30	10	21	17	19	20	32	26	26	21.5	22.5
Average....	76	38	8	18	14	15	14	25	21	24	17	19.4

monary vein was one mm. of Hg higher than through that obstructing the pulmonary artery. The mean pressures recorded by the saline manometer were in close agreement with the mean pressures obtained with the Hamilton manometer.

In one experiment, *dog 82*, catheters were wedged fortuitously into the pul-

monary artery and vein in the same part of the lung. Fluoroscopically, they appeared in juxtaposition when viewed in the right anterior oblique position. A rapid injection of I cc. of saline through either catheter produced a sharp rise of over 50 mm. Hg of pressure in the catheter wedged into the vessel on the opposite side of the capillary bed (fig. 3), indicating not only a free communication between the two but also the relatively small volume of the intervening capillary bed.

When the pressure in the pulmonary artery was raised by the injection of lycopodium spores through a third catheter located in the artery (4) of the opposite lung,

TABLE 2. VARIATIONS IN PRESSURE IN PULMONARY ARTERY AND IN OBSTRUCTED PULMONARY ARTERY IN 18 NORMAL DOGS UNDER NEMBUTAL ANESTHESIA

Values in mm. Hg

EXP. NO.	PRESSURES WITH CATHETER IN					
	Pulmonary artery		Pulmonary end artery			
	Hamilton manometer		Hamilton manometer			Saline manometer mean
	Syst.	Diast.	Inspir.	Expir.	Mean	
86	21	7	2	4	3	3
88	30	8	0	5	3	2
89	28	8	−5	9	2	2
90	17	8	−1	9	4	3
91	30	12	−2	9	4	5
92	25	6	1	5	3	3
93	35	12	0	13	8	9
94	20	8	0	9	5	1
95	31	14	3	11	6	6
96	30	3	0	6	4	3
97	20	7	0	6	4	7
99	28	9	0	6	4	4
100	21	6	−5	7	2	2
101	23	3	0	4	3	3
102	32	12	0	8	5	7
103	—	—	−2	6	2	3
104	24	9	2	4	3	6
105	22	8	−2	5	2	2
Average...	26	8	0	7	4	4

pressures recorded through the catheters wedged into the pulmonary artery and vein eventually became elevated but maintained their same general relationship, i.e. were higher on the pulmonary venous than on the pulmonary arterial side (table 1).

To determine the effect of respiration on the two pressures, two experiments were done in which the animal was maintained on artificial respiration after the spinal cord was pithed. Under artificial respiration, pressures recorded with the catheter in the pulmonary vein were higher than those recorded in the pulmonary artery. When the artificial respiration was stopped for one minute, both pressures fell about 2 cm. of water (fig. 4). The pressure through the catheter obstructing the pulmonary vein, however, continued to be higher than that recorded through the catheter obstructing

the pulmonary artery.   Immediately after the heart failed to show electrical activity by electrocardiogram and therefore the flow of blood had ceased, the pressures measured on the two sides of the capillary bed became equal (if corrected so that the tips of the catheters were at the same hydrostatic level).   The induction of artificial respiration in this dead animal caused a slight increase in both pressures, but this rise in pressure was of the same magnitude in each.

In the 6 animals in which the catheter was passed through the left side of the heart, 4 showed damage consisting of small endocardial hemorrhagic areas on the mitral and aortic valves and in one animal there was a subendocardial hemorrhage in the wall of the left ventricle.

The catheter which obstructed the pulmonary artery for one to four hours produced no changes suggestive of infarction of the lungs.
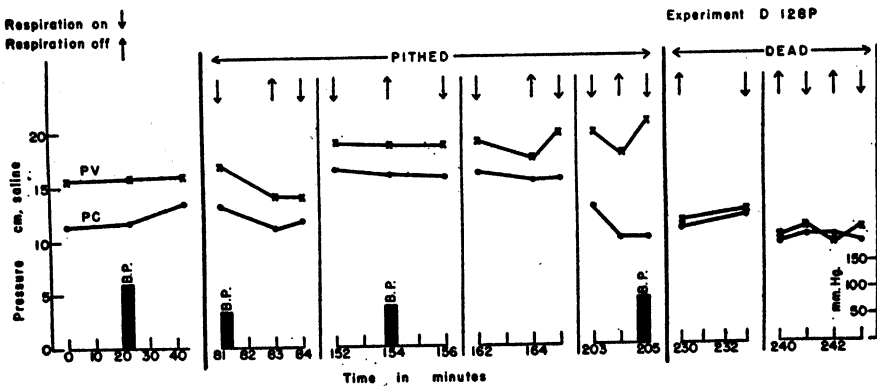
Fig. 4. EFFECT of artificial respiration on pressures recorded through catheters obstructing pulmonary artery (P.C.) and pulmonary vein (P.V.).
Note 1) that pressure on the venous side is almost uniformly higher than that on the arterial side; 2) that when blood flow ceases (death), the pressures fall to essentially the same level indicating that (1) above is an artifact due to blood flow; and 3) that when respiration is induced, both pressures increase slightly (pumping action of the lung).

DISCUSSION

Anatomical studies of the vessels of the lung by histological and injection techniques have indicated the following features which are pertinent to the present study. The pulmonary artery undergoes a series of subdivisions finally ending in a rich plexus of freely anastomosing capillaries.   Bronchial arteries likewise subdivide and end in capillaries which anastomose freely with the capillaries of the pulmonary artery.   In a review of the literature and from his own observations, Miller (6) concluded that there are normally no anastomoses between the bronchial and pulmonary arteries proximal to the pulmonary capillary bed.   Bruner and Schmidt (7) have also arrived at the same conclusion.   Physiologically the same has been demonstrated (8) in that blood, fully saturated with oxygen, may be withdrawn through a catheter wedged into a branch of the pulmonary artery of patients thus indicating that the catheter was in direct communication with the capillary bed and that any significant number of precapillary anastomoses between different branches of the pulmonary artery did not exist.   If such were present, this blood would be unsaturated.   In patients with

arterial oxygen unsaturation, blood withdrawn in a similar fashion has been shown to be fully saturated with oxygen, indicating the absence of any significant precapillary anastomoses between systemic (bronchial and intercostal) arteries and pulmonary artery (9).

Anatomically there are no valves in the pulmonary vessels (6). Dyes injected into a pulmonary vein in a retrograde direction flow into the pulmonary capillaries and then into the pulmonary and bronchial arteries (10). These observations are confirmed in the present study in which catheters were wedged into a pulmonary artery and vein in close approximation one to the other. A quick injection of only 1 cc. of saline into either catheter produced an abrupt rise of pressure in the opposite catheter on the other side of the capillary bed. This indicated *in vivo* a free communication in both directions between pulmonary artery and pulmonary vein without valvular or other gross obstruction and supplied indirect evidence that the obstructing catheter was in communication with only a small area of the capillary bed.

In view of these anatomical relationships, the pressure recorded distal to a point of obstruction in the pulmonary artery or vein should not be zero since an anastomotic circulation exists. It should theoretically be the pressure in the next collateral branch but on the arterial side blockage may lessen the measured pressure by reducing flow locally, just as on the venous side similar blockage may raise pressure by local passive congestion.

Gomez (11) has observed that when a systemic end artery is occluded, the pressure distal to the point of occlusion falls, not to zero, but to a point considerably above the pressure in the corresponding vein. This he refers to as the 'static pressure' of the artery. Starr and Rawson (12, 13) have likewise studied static pressure in veins. Since blood flow was maintained through all parts of the lung except in the one vessel obstructed by the catheter, static pressure could not be measured under the circumstances of these experiments.

By occluding a branch of the pulmonary artery with a catheter and a branch of a pulmonary vein in a different region with an arterial catheter, a direct connection with the capillary side of each vessel was maintained through the hole on the tip of the catheters. In this way, pressures on each side of the capillary bed, with the main vessel occluded, could be measured.

Under the conditions of these observations, pressures recorded in the obstructed pulmonary artery were lower than those recorded in the obstructed vein. This difference in pressures was not abolished by stopping respiration although there was a slight inconstant reduction in their levels when respiratory movement ceased. Immediately after blood flow had stopped (death), these two pressures became equal. Artificial respiration on these dead animals caused a slight rise of pressure in both. Therefore the differences are related largely to blood flow and only slightly to the pumping action of the lung. The discrepancy in the two pressures is probably due to the relationship of the catheters to the capillary bed, i.e. the pressure recorded through the obstructed artery is less because blood flow is reduced locally and that through the obstructed pulmonary vein is greater due to passive congestion.

It is believed, therefore, that the pressure recorded on the pulmonary artery side of the capillary bed is probably no greater than a few millimeters of mercury below

the true pulmonary capillary pressure and that on the pulmonary venous side is probably no greater than a few millimeters of mercury above the true pulmonary capillary pressure. With this reservation, pressures on the arterial side can be used to measure indirectly the direction and approximate magnitude of changes in capillary pressure. Averaging of both figures (table 1) where both can be obtained as in animals gives a means of estimating the absolute magnitude of capillary pressure.

In recording the pressure with a column of saline, the fluid runs through the catheter into the capillaries, until equilibrium is established. In recording the pressure with a Hamilton manometer, the pressure of the blood in the pulmonary capillaries is transmitted backward through the lumen of the catheter and to the membrane of the manometer. These two methods of measurement should be in close agreement. Any marked discrepancy as occasionally occurs indicates an obstruction between the catheter and the capillaries. In such circumstances, the recordings are inaccurate and should be discarded. However, it is felt that the Hamilton manometer, in general, gives the more accurate reading as equilibrium is obtained immediately while with the saline manometer equilibrium is slowly reached.

It should be pointed out that the pressures recorded in the manner described were related to a point 7.5 cm. anterior to the animals' spine when lying in the recumbent position. No attempt was made to relate the zero point to the level of the tip of the catheter which would be necessary in the estimation of the true hydrostatic pressure in the capillaries. In the majority of the dogs, however, the tip of the catheter lay about in the middle of the chest when viewed in the lateral position fluoroscopically.

It is considered that the damage found in the chambers of the left side of the heart definitely prohibits the use of arterial cardiac catheterization in man.

SUMMARY

1. A venous catheter was introduced into the pulmonary artery of dogs and wedged into a distal ramification so as to obstruct its lumen. Pressures beyond the point of obstruction were recorded through a hole in the tip of the catheter with a Hamilton manometer and with a saline column.

2. An arterial catheter was introduced by way of the carotid artery and left side of the heart into a pulmonary vein in similar fashion and pressures were similarly recorded.

3. In dogs, the pressures recorded with a Hamilton manometer through the catheter blocking the pulmonary artery varied between 5 and 8 with an average of 6 mm. Hg while those measured simultaneously through the catheter blocking the pulmonary vein varied between 6 and 14 with an average of 10 mm. Hg (table 1). The former is probably lower than true pulmonary capillary pressure by the 'velocity factor' and the latter too high by this same factor. It is suggested that true mean capillary pressure with unobstructed flow normally lies between these two figures, the average in 6 animals varying from 5.5 to 10 mm. Hg with an average of 8.3 mm. Hg.

4. It is considered that damage to the left side of the heart from arterial catheterization prohibits its use in man.

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HEMORRHAGIC HYPOTENSION IN HEPATECTOMIZED AND BILATERALLY NEPHRECTOMIZED HEPATECTOMIZED DOGS[1]

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INVESTIGATIONS of hypotensive states naturally led to the study of the contributions of the kidneys and liver in the maintainance of normal blood pressure. A participation of the renal pressor system was indicated by Sapirstein, Ogden, and Southard (1, 2), Hamilton and Collins (3) and Huidobro and Braun-Menendez (4) whose observations implied a homeostatic action of renin on the arterial pressure after bleeding. A function of the liver in shock was emphasized by Fine, Seligman, and Frank (5, 6) who concluded that inadequate hepatic blood flow is an essential factor in irreversible hemorrhagic shock. Shorr, Zweifach and Furchgott (7) described what seemed to be an integrated pattern of hepatic and renal responses to hemorrhage consisting in renal liberation of a protective VEM (vaso-excitor material) and hepatic release of an opposing principle VDM (vaso-depressor material).

As a more direct approach, these organs were individually and jointly removed before prolonged periods of hypotension were induced by controlled hemorrhage. The blood removed to produce hypotension was later reinfused to observe the ability of these animals to regain normal arterial blood pressure. Another group of animals was hepatectomized and transfused with blood from dogs which had previously endured periods of prolonged hypotension due to hemorrhage.

METHODS

Hepatectomy was performed under light ether anesthesia by the one-stage method of Firor and Stinson (8). The animals were given intravenously 250 cc. of 10 per cent glucose in physiologic saline and 125 cc. of normal dog blood during the operation. Following recovery from ether, glucose was supplied intravenously in doses of 0.25 gm. per kilogram body weight per hour (9) and the animals were not again anesthetized. In experiments on bilaterally nephrectomized, hepatectomized animals, the nephrectomies were performed first and immediately followed by one-stage hepatectomy. Since all the experiments were of short duration, the operations were done without sterile technique.

Following a 1½-hour recovery period, experimental hemorrhagic hypotension was produced by the method of Wiggers (10) and Kohlstaedt and Page (11) and the stage of shock estimated from the criteria of Glasser and Page (12). In brief, the animals were bled rapidly from a femoral artery into a reservoir containing heparin until arterial pressure of 50 mm. Hg was established. Pressure was maintained in the reservoir by a hand bulb connected with an aneroid type manometer. The shed blood in the reservoir was in open communication through rubber tubing with the femoral

artery of the test animal. Consequently, if the arterial pressure of the animal fell below the 50 mm. Hg level, blood passed from the reservoir into the dog. Conversely, if the arterial pressure of the animal tended to rise, more blood was shed by the dog into the reservoir. The 50 mm. Hg pressure was continued for at least 90 minutes, when the pressure was lowered to 30 mm. Hg by decreasing the pressure in the reservoir and allowing more blood to flow into it from the animal. This new pressure level was maintained for 45 minutes. Following the successive phases of hypotension, blood was restored to the animal by stepwise increases of pressure in the reservoir to the control level. Kymographic records (fig. 1) were made of arterial pressure (A.P.), shed blood volume (B.V.), respirations (RESP.) and responses to epinephrine (A.) (fig. 1). Application of this method has recently been made to a large number of animals in a critical study of hemorrhagic shock by Glasser and Page (13).

In the transfusion experiments, donor animals were anesthetized intraperitoneally with sodium pentobarbital, 30 mg./kg. body weight. The donors were similarly subjected to shock and intra-arterial reinfusion. Their blood was then drawn and citrated for transfusion into hepatectomized recipients. Blood was drawn in equal volume from the opposite legs of the recipients during the transfusion so that blood volume was unchanged. The recipient hepatectomized animals were observed for possible deleterious effects of transfusion. They were then subjected to the usual period of hemorrhagic hypotension. All animals were tested with standard doses of intravenously administered epinephrine and the rise in blood pressure was referred to as epinephrine response and expressed in mm. Hg. Page (14) has suggested that among other criteria the magnitude of this response is a rough measure of the phase of shock existing in animals with hypotension.

<center>RESULTS</center>

Animals subjected to hepatectomy invariably had widely varying degrees of blood loss into the peritoneal cavity, apparently due to diapedesis through the peritoneum. This blood loss could not be expeditiously measured pre-mortem. Consequently, among other factors, the amount of bleeding necessary to produce a standard hypotension varied widely among different experiments.

A 1½-hour recovery period followed the procedures of hepatectomy and hepatectomy-nephrectomy. The hemorrhagic hypotension lasted 140 minutes. Survival for all groups averaged 7 hours after hepatectomy and 2.5 hours after hypotension.

Group I. Hemorrhagic Hypotension in Hepatectomized Dogs. Seven experiments were performed in which hepatectomized dogs were bled to produce hypotension. The volume of blood withdrawn to decrease the pressure to the 50 mm. Hg level varied from 20 cc. to 40 cc. per kilogram body weight. Five animals were within the narrow range of 27 cc. to 33 cc. and two, 19 cc. and 40 cc. per kilogram. Blood flowed from the reservoir into the femoral arteries of several animals in order to maintain the 50 mm. Hg level. These inflows varied and are listed in table 1A. All animals survived the procedure and regained normal blood pressure levels after the intra-arterial reinfusion of shed blood. In one case 225 cc. of additional 5 per cent gelatin solution besides the shed blood were necessary to restore the pressure to normal. The epinephrine responses in the pre- and post-hypotensive periods were equal.

Survival of these animals was limited by the nature of the experiment; however, they had normal arterial pressures for periods of from 1½ to 4½ hours. Two animals required further intra-arterial infusion of 375 cc. and 175 cc. of 5 per cent gelatin solution during these post-hypotensive periods, but again, the peritoneal loss was unknown. The results are summarized in table 1A. A typical kymographic tracing is shown in figure 1.

Group II. Hemorrhagic Hypotension in Bilaterally Nephrectomized Hepatec-tomized Dogs. There were 6 dogs in this group. The procedure after nephrectomy and hepatectomy exactly followed that used in *Group I.* The amount of bleeding

TABLE 1. HEMORRHAGIC HYPOTENSION

DOG NO.	BLOOD WITHDRAWN TO PRODUCE HYPOTENSION	INTAKE AT 50 MM. HG	INTAKE AT 30 MM. HG	AMT. INTRA-ART. BLOOD REPLACEMENT	SUPPL. FLUID REINFUSION 5% GELATIN	SURVIVAL POST HEPATEC-TOMY	POST HYPOTEN-SION
	cc/kg.	cc/kg.	cc/kg.	cc/kg.	cc/kg.	hrs.	
A. In Hepatectomized Dogs							
1	28	1	4	19	30[1]	8.5	4.25
2	30	0	0	26	0	7.5	3.5
3	33	0	7	26	20[1]	7.0	3.5
5	19	11	15	19	22[2]	6.5	3.0
13	27	6	3	18	0	5	1.5
14	40	2	4	24	0	7.0	2.75
15	28	5	7	12	7[3]	7.0	3.0
B. In Bilaterally Nephrectomized-Hepatectomized Dogs							
4	11	0	0	11	22[2]	6.5[4]	3.0[4]
6	19	6	0	19	23[2]	5.5[4]	2.0[4]
20	31	0	0	26	0	6.5[4]	3.0[4]
17	31	0	4	27	0	6.5[4]	3.0[4]
16	27	2	0	27	0	5.5[4]	3.25[4]
18	22	3	1	17	0	7.0	1.5

[1] Infused intra-arterially in post-reinfusion period.   [2] Infused intra-arterially during hypotensive periods.

[3] Blood infused in post-reinfusion period.   [4] Killed.
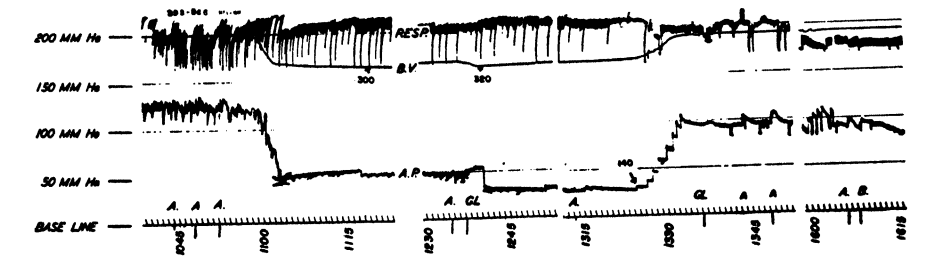
Fig. 1. Kymographic record of *dog 86-6* (Sept. 16, 1947): *RESP*, respiration; *BV*, blood volume cc. in reservoir, with base line at 200 mm. Hg; *A.P.*, arterial pressure; time intervals of 1 min.; *A*, 0.1 cc. 1–10,000 epinephrine; *GL*, glucose 0.25 gm., 1 kg/hour intravenously; *B*, BaCl$_2$ 9 mg. intravenously. Hepatectomy performed at 9:30 A.M. Duration of 50 mm. hypotension, 90 min. and of 30 mm., 50 min.

necessary to produce the required hypotensive levels varied from 11 cc. to 31 cc. per kilogram body weight. Four animals were within the range of 22 cc. to 31 cc. The other two shed 11 cc. and 19 cc., respectively. Three animals took blood from the reservoir in order to maintain pressures of 50 mm. Hg. These values are listed in table 1B. All survived the periods of hypotension and were sacrificed 1½ to 3 hours after reinfusion. The blood pressure in each was restored to normal levels after

intra-arterial reinfusion of shed blood. Two received 200 cc. of additional 5 per cent gelatin solution. The responses to 0.1 cc. and 0.2 cc. of epinephrine 1-10,000 were the same in the pre- and post-shock periods. One animal required 150 cc. of 5 per

TABLE 2. HEMORRHAGIC HYPOTENSION IN HEPATECTOMIZED DOGS

DOG NO.	AMT. BLOOD TRANSFUSED FROM SHOCKED DOGS	BLOOD WITHDRAWN TO PRODUCE HYPOTENSION	INTAKE AT 50 MM. HG	INTAKE AT 30 MM. HG	INTRA-ART. BLOOD REPLACEMENT	AMOUNT SUPPL. FLUID REINFUSION	SURVIVAL POST HEPATECTOMY	POST HYPOTENSION
	cc/kg.	cc/kg.	cc/kg.	cc/kg.	cc/kg.	cc/kg.		
A. Following Transfusion from Intact Shocked Dogs								
7	16	3	3	3	15	13^1	5.25	1.25
8	25	13	0	3	13	0	5.25^2	1.5^2
23	23	28	7	3	27	14^1	5^2	2.0^2
19	27	40	0	7	32	0	5^2	2.0^2
25	21	19	2	8	19	3^1	5.5^2	1.5^2
24	40	13	5	3	11	0	5^2	1.0^2
B. Following Transfusion from Bilaterally Nephrectomized Dogs Subjected to Hemorrhagic Hypotension								
10	32	17	2	2	17	29^3	5^2	1.0^2
11	27	8	13	5	8	27^3	6	1.25
12	20	20	2	0	20	8^3	5^2	1.0^2
21	18	39	5	4	33	0	5^2	2.0^2
22	20	21	4	0	21	6^1	5^2	1.5^2
9	27	18	2	3	18	10^3	$5\frac{1}{2}^2$	1.5^2

[1] Blood infused intra-arterially during hypotensive periods.   [2] Killed.
[3] Blood infused intra-arterially during post-reinfusion periods.
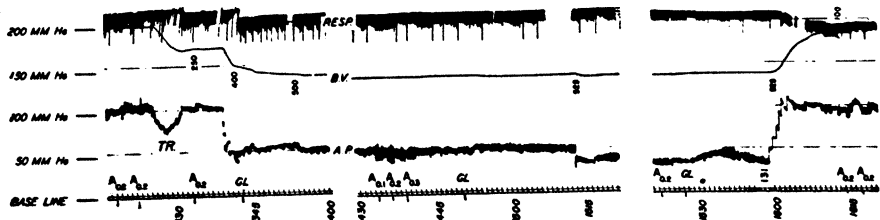
Fig. 2. Kymographic record *dog 95-1* (Nov. 13, 1947): *RESP*, respiration; *B.V.*, blood volume cc. in reservoir, with base line at 200 mm. Hg; *A.P.*, arterial pressure; time intervals of 1 min.; A, epinephrine 1-10,000 expressed in cc.; *GL*, glucose 0.25 gm/kg. hour intravenously. Experimental procedures: hepatectomy performed at 11:00 A.M.; *TR* transfusion of 300 cc. blood from bilaterally nephrectomized shocked dog with simultaneous removal of 300 cc. of blood from opposite leg of *dog 95-1* at 1325 (1:25 P.M.). Duration of 50 mm. hypotension 90 min. and 30 mm., 45 min.

cent gelatin during the post-hypotensive period in order to maintain a normal blood pressure. The results are summarized in table 1B.

    Group III. Hemorrhagic Hypotension in Hepatectomized Dogs Following Transfusion from Shocked Dogs with Intact Livers. Six hepatectomized and 6 normal donor animals were included in the third group of experiments. Each donor animal was bled into hypotension of great enough duration to have a poor prognosis for survival

according to the criteria of Glasser and Page (5). After this was established, shed blood was replaced by intra-arterial transfusion with a pressure in the reservoir great enough to maintain normal blood pressure 10 to 20 minutes. These animals were then bled to death. The volumes so obtained (160 cc.–500 cc.) were transfused intravenously into the hepatectomized recipients while equivalent amounts of blood were withdrawn. According to our observations, these procedures had no effect upon the liverless recipients. These animals were then subjected to hypotension induced by hemorrhage in a manner similar to that of the preceding two groups. The results were not appreciably different and are recorded in table 2A.

Group IV. Hemorrhagic Hypotension in Hepatectomized Dogs Following Transfusion from Bilaterally Nephrectomized Hypotensive Dogs. This group of experiments was a repetition of those in *Group III* save that the 6 donor animals had been previously nephrectomized. The 6 hepatectomized recipient animals again gave no evidence of being affected by this exchange of blood. Likewise, induction of the standardized hypotensive state caused no fatalities during its course, and the arterial pressure was normal in all of the animals after intra-arterial reinfusion of the shed blood. These results are summarized in table 2B. A typical kymographic tracing is presented in figure 2.

DISCUSSION

A variety of altered physiological reactions—neurogenic, humoral and hemodynamic—appear during hemorrhagic hypotension. This study deals with humoral and hemodynamic factors of renal and hepatic origin since conditions of our experiments were so arranged that the neurogenic influences on the hypotensive state were believed minimal and reasonably constant.

Vasoconstriction is now generally recognized as prevailing during the onset of shock. It was attributed by Page (15) to the action of vasoconstrictor material originating in injured tissue and to vasoconstrictor impulses. Further, the caliber of the vessels seems to be indirectly influenced by their reactivity to chemical stimuli. When the responsiveness of the vascular tree is estimated by the pressor responses to epinephrine in burn shock, three phases can be distinguished: 1) the injury phase with increased arterial pressure and variably increased pressor response; 2) a transitional phase of increased pressor response followed by progressive decrease; and 3) the terminal phase in which vascular responsiveness to pressor and depressor agents is severely reduced or absent. The initial injury phase was absent in shock due to bleeding.

More recently, Shipley, Helmer and Kohlstaedt (16) have described a pressor principle which causes sustained elevation of the arterial pressure of nephrectomized, pithed cats. It is present in the plasma of sick cats, of cats poisoned with DDT and of animals subjected to hemorrhagic hypotension. Since they could not detect it in nephrectomized animals subjected to the same procedure, they consider it of renal origin. Shipley and Helmer (17) further demonstrated that the pressor principle gives only brief, unsustained increases in blood pressure in intact cats, while the responses in cats nephrectomized 48 hours before testing were invariably sustained

for several hours. These observers suggest that an inhibitor of the pressor principle is secreted by the kidneys into the blood and its concentration in blood diminishes progressively after nephrectomy. They also demonstrated that injections of pressor principle neutralizes the circulating inhibitor in recently nephrectomized animals so that repeated injections finally produce sustained responses similar to those of the 24- or 48-hour nephrectomized animals.

Hamilton and Collins (3) have shown that hemorrhage imparts pressor activity to blood as tested on nephrectomized dogs. The homeostatic capacity of renal pressor agents in shock is not large, for Bobb (18) found that nephrectomy had no effect on mean arterial pressures, survival times or rectal temperatures in dogs subjected to tourniquet shock.

| PRE-HYPOTENSIVE (Control) B.P. | POST-REINFUSION   B.P. |

● — Hepatectomized dogs
■ — Hepatectomized, Nephrectomized dogs
□ — Hepatectomized dogs transfused from intact shocked dogs
○ — Hepatectomized dogs transfused from nephrectomized shocked dogs
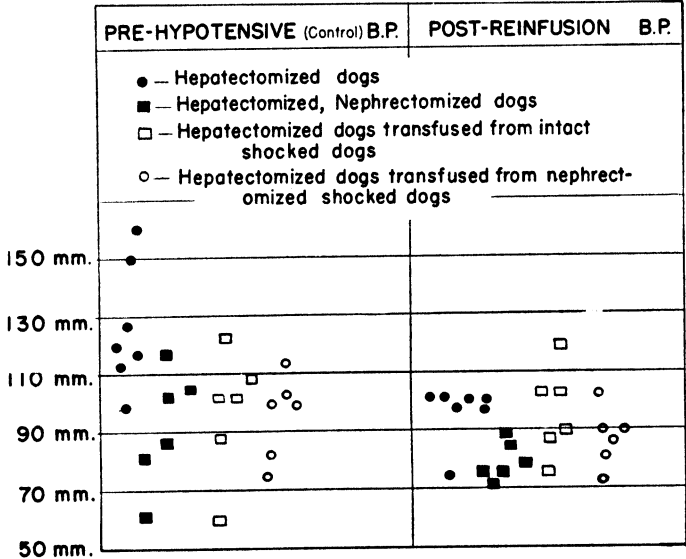
FIG. 3. GRAPH of pre-hypotensive (control) arterial blood pressures and post-reinfusion pressures of *Groups I, II, III* and *IV*.

Frank, Seligman and Fine (5, 6) concluded that loss of liver function is of crucial importance in the collapse of the organism in advanced hemorrhagic shock. These workers were able to prevent irreversible shock by vivi-perfusion of the liver of a shocked dog with the blood of a normal one. They go so far as to suggest that this is the essential effective treatment of 'irreversible' hemorrhagic shock.

Thus a summary of recent investigations indicates that *a*) experimental shock exhibits an early compensatory phase associated with vasoconstriction and frequently with increased vascular responsiveness followed by a late decompensatory phase associated with vasodilatation (Page and Abell, 13) and loss of pressor responsiveness; *b*) vasoconstrictor or vasoexcitor materials are present in the circulating blood of shocked animals; *c*) the kidney is the site of origin of a sustained pressor material on the one hand, and possibly of a distinct vasoexcitor substance (VEM) as well as a

source of anti-pressor material; and *d*) the integrity of the liver is considered absolutely necessary if the organism is to withstand shock. Our observations stand in contrast to some of these views.

We believe it desirable to avoid the terms 'shock' or 'irreversible shock', as they apply to hepatectomized animals, while they do not in any case long survive. We shall use instead the term 'hemorrhagic hypotension', as defined above under METHODS.

Regardless of whether the liver or both liver and kidneys were removed, approximately equivalent amounts of hemorrhage were necessary to lower the arterial blood pressure to the selected levels of 50 mm. Hg for 90 minutes and 30 mm. Hg for 45 minutes. Further, the bleeding volumes of hepatectomized animals, which received large volumes of blood from intact animals and from nephrectomized animals in hemorrhagic hypotension, did not differ greatly from those of hepatectomized and hepatectomized-nephrectomized animals which were not subjected to transfusion. The fact which stands out is that these animals withstand varied procedures of hemorrhage and transfusion from animals in hemorrhagic hypotension, intact or nephrectomized. Further, appropriate transfusion of shed blood restores arterial pressure and epinephrine pressor responsiveness (fig. 3).

It was apparent from 16 dogs that if only the blood removed or less were restored to the animals intra-arterially, a normotensive state could be re-established. In 9 dogs additional fluid was necessary, both during the hypotensive states and after reinfusion. The significance of this supplement cannot be established inasmuch as we have no measure of the amount of blood lost by diapedesis into the peritoneal cavity at the time of reinfusion.

SUMMARY

Animals deprived of livers or livers and kidneys can be subjected to prolonged hemorrhagic hypotension and still retain the ability to re-establish and maintain for some time normal arterial pressure following intra-arterial reinfusion of shed blood. This ability is not impaired by exchange transfusion of hepatectomized animals with blood from animals in hemorrhagic hypotension both intact and nephrectomized. Thus, these direct experiments do not demonstrate a critical function of the liver or kidneys in the vascular response to a standardized hemorrhagic hypotension.

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EFFECTS OF REPEATED ORAL DOSES OF QUININE AND QUINIDINE ON THE BLOOD PRESSURE AND RENAL CIRCULATION OF DOGS WITH EXPERIMENTAL NEUROGENIC HYPERTENSION[1]

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IT HAS been shown in this laboratory that single oral doses of the cinchona alkaloids will cause an increase in renal blood flow and glomerular filtration rate in normal dogs (7). This effect lasts for several hours and occurs without significant change in arterial blood pressure. In the investigations reported in this paper we have studied the effect of repeated oral doses of quinine and quinidine given over a period of several days on the circulation of normal dogs and of dogs with experimental neurogenic hypertension.

METHODS

A sustained hypertension was obtained in 4 dogs by excision of both carotid sinuses and division of the cervical vago-depressor-sympathetic nerve trunk on one side and the depressor nerve of the opposite side, according to the technic of Bouckaert as described by Grimson (6). These dogs, together with several normal dogs, were observed before, during and after a period of several days in which they received two to four daily doses of 10 to 15 mg./kg. of quinine or quinidine sulfate. The experiments were carried out at different intervals varying from one to 16 months after the surgical operation.

Mean arterial blood pressure was measured by puncture of the femoral artery with a 20-gauge hypodermic needle connected through a tube filled with 5 per cent sodium citrate solution to a mercury manometer. One per cent procaine without epinephrine was injected into the tissues around the artery before the puncture was made. Readings of pressure were taken a minute or two after the needle was introduced, when the pressure seemed to be fairly steady.

The renal clearance of sodium p-aminohippurate (PAH) was determined as a measure of the effective renal plasma flow. The creatinine clearance was determined as a measure of the glomerular filtration rate. PAH concentrations in plasma and urine were determined by the method of Smith *et al.* (9) while creatinine concentrations were analysed by the alkaline picrate method of Folin and Wu (5). These agents were administered subcutaneously to obtain satisfactory plasma concentrations.

Quinine and quinidine concentrations in the plasma were determined by the method of Brodie and Udenfriend (3).

It was our usual practice to give four doses the first day and three doses on subsequent days with a 10-hour interval between the last dose of one day and the first dose of the next day. After the control observations, most of the measurements were made before the first dose of the day, at the time when plasma concentrations were at the daily minimum, but occasional measurements were made at shorter intervals after a dose of alkaloid. The observations were continued for at least two days after the drugs had been discontinued.

RESULTS

The results of our experiments on all 4 dogs were essentially the same and are indicated in the accompanying graphical summaries of typical experiments (fig. 1).

a. *Plasma Alkaloid Concentrations.* Most of these fell within the range of 1 to 4 mg/l. These levels were effective in causing the changes described below but were not toxic as indicated by the normal behavior and appetite of the dogs. After a few doses the plasma concentration was well maintained between doses, even during the 10-hour overnight period. Two doses a day did not maintain plasma concentrations in the effective range, but three doses a day seemed to be adequate.
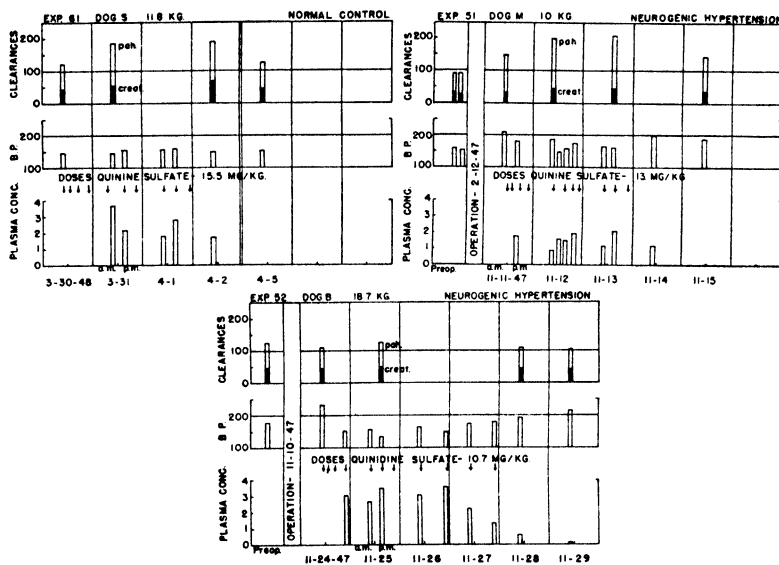
Fig. 1. GRAPHICAL SUMMARIES of three typical experiments with repeated oral doses of cinchona alkaloids. Renal clearances are expressed in cc. of plasma/min. The blood pressure is recorded as mm. of Hg mean femoral artery pressure. Plasma concentrations are shown in mg/l. of plasma. The vertical lines indicate time in days with the dates recorded on the abscissa. *A. (upper left)* Effect of repeated doses of quinine sulfate on a normal dog. *B. (upper right)* Effect of repeated oral doses of quinine sulfate on a dog with neurogenic hypertension. Preoperative data are shown on the column on the left. *C. (lower)* Effect of repeated oral doses of quinidine sulfate on another dog with neurogenic hypertension.

b. *Blood Pressure.* In the normal dogs there was little change in the mean arterial pressure. In the dogs with neurogenic hypertension there was a marked fall in blood pressure, in most cases closely approaching their normal preoperative pressures. After the second day of administration of the alkaloids the depressor effect was well maintained between doses. When the drugs were discontinued the blood pressure gradually returned to the original hypertensive levels as the plasma concentrations decreased. This recovery was usually complete in about 36 hours. Quinidine had a more marked effect on the blood pressure than quinine.

c. *Renal Circulation.* In most of the normal dogs receiving repeated doses of cinchona alkaloids, as in the experiments with single doses reported previously

(7), the renal plasma flow and the glomerular filtration rate increased, the former more than the latter. In most instances the effect lasted during the period of administration of the drug. Unlike the single dose experiments (7), the present data, with repeated doses, shows that quinine causes a greater renal hyperemia than quinidine.

Essentially the same effects on renal circulation were observed in hypertensive dogs. In spite of a considerable fall in arterial blood pressure there was no decrease in renal plasma flow and in most instances there was a definite increase, especially with quinine.

d. *Heart Rate.* In most of the normal dogs an increase in heart rate occurred after administration of the alkaloids. The hypertensive dogs which maintain a rapid heart rate (10) showed either a further acceleration of the rate or no change under the influence of the drugs.

DISCUSSION

In considering the mechanism by which the cinchona alkaloids reduce the blood pressure of our dogs with neurogenic hypertension there is not much help to be found in the literature. Most of the reported experiments have dealt with the depressor effect of the alkaloids injected intravenously (which is much more drastic than when the same amount of alkaloid is administered orally) and the plasma concentration of the drugs was not measured. There was, and still is, a controversy over whether or not most of the depressor effect is due to cardiac depression or to peripheral vasodilatation. Nelson (8) who reviewed the old literature felt that the action of these alkaloids is due to peripheral vasodilatation. He thought this action to be due in part to depression of the vasomotor endings and in part to an action directly on the smooth muscle of the blood vessels.

Nelson also demonstrated the antagonism of quinine and quinidine for the circulatory effects of epinephrine. We have succeeded in verifying this in experiments which will be reported later. Dreisbach and Hanzlik (4) have studied the converse relationship, i.e., the antagonism by epinephrine of the depressor effects of intravenous quinine.

Bing, Thomas and Waples studying the circulation of dogs with experimental neurogenic hypertension found evidences of increased sympathetic tone as indicated by increased cardiac output, increased renal vascular resistance and increased blood flow through the forelimb (2). These investigators also studied the effects of the adrenolytic dioxane derivatives 883 and 933 F on dogs with neurogenic hypertension and found a delayed fall in blood pressure due largely to a diminished cardiac output with a decrease in heart rate (1).

All we can say definitely at this time is that these cinchona alkaloids cause a sustained decrease in blood pressure in dogs with neurogenic hypertension. At least part of their effect is due to vasodilatation as indicated by the renal hyperemia and the well-known cutaneous flushing (8) which follows the administration of these drugs. Independent investigation of possible effects of these drugs on cardiac ouput are currently being undertaken.

SUMMARY

Oral doses of quinine or quinidine of the order of 10 to 15 mg/kg. administered to dogs three times a day for several days had the following effects: *a*) in normal dogs a sustained renal hyperemia (as measured by clearance methods) without much change in blood pressure; *b*) in dogs with neurogenic hypertension, a sustained fall in the blood pressure without any decrease in renal circulation. With quinine there is actually an increase in renal blood flow. Quinidine had a greater depressor effect on the blood pressure than quinine. These effects were achieved with plasma concentration of cinchona alkaloid in the range of 1 to 4 mg/l.

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BLOOD PRESSURES OF RATS SUBJECTED TO AUDITORY STIMULATION[1]

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RESULTS published by Medoff and Bongiovanni (1) indicated that a significant increase in systolic blood pressure, as measured by Griffith's method (2), occurred in gray Norway rats after prolonged exposure to the sound of a blast of compressed air. In view of the inference that this procedure might be a means of inducing a neurogenic elevation of blood pressure in response to an exteroceptive stimulus, we were led to repeat the experiment, using, however, a different method of measuring pressures in order that readings might be made as frequently as desired and without the use of anesthesia. In addition, direct measurements of systolic and diastolic pressures were obtained in a number of the animals under light ether anesthesia.

MATERIAL AND METHODS

The rats were domesticated gray Norways of the Wistar Institute colony, ranging in age from 5 months to a year at the start of the experiment. Nine male and 8 female controls and 12 male and 13 female experimental rats were used. The control and the experimental groups were divided as equally as possible according to litter and sex. When there was a choice between rats with differing blood pressures, as shown by preliminary measurements, the animal with the higher pressure was put in the control group.

The method of air blasting has been described elsewhere (3). All experimental animals were exposed to the sound for 5 minutes a day, 5 days a week for 12 months. Blood pressures were taken during 2 months before air blasting and at random intervals thereafter, more frequently in the experimental rats and in the later stages of the experiment.

A tail plethysmograph, embodying modifications advocated by Shuler, Kupperman and Hamilton (4), was used. Following the suggestion of these authors the rats were not heated prior to testing but it was found helpful, as shown by Sobin (5), to warm the water in the apparatus. The animals were held in a cylindrical wire mesh cage of suitable diameter with a plunger at one end. The plunger was adjusted to accommodate the rat so that the full length of the tail protruded from the opposite end of the cage and could be inserted to the root in the plethysmograph. As an aid in keeping the animal quiet, a black cloth was wrapped around the head end of the holder. No anesthetic was used at any time.

Blood pressures were determined in the following way. Three consecutive readings were made, and if they agreed within 10 mm. Hg the average was taken as the blood pressure of the rat for that day. If the variation of the readings was greater, measurements were continued until agreement within a range of 10 mm. was reached. Occasionally, despite repeated trials, any three consecutive

readings might vary as much as 20 mm. if the animal was restless.   The averages in such instances were compared with readings of the rat's blood pressure on other days when the variation fell within the prescribed limits and, if found to correspond closely, the former figures were included in the data. The number of daily blood pressures used in calculating the mean blood pressure of a rat over a given period of time is indicated on the tables in *column N*.

Direct blood pressure measurements under light ether anesthesia were obtained with a Hamilton optical manometer[3] on 11 of the control and 13 of the experimental rats.

TABLE 1.   AVERAGE SYSTOLIC BLOOD PRESSURES OF RATS AT BEGINNING AND END OF EXPERIMENT

	CONTROL RATS					EXPERIMENTAL RATS				
Rat and sex	2-month 'Control Period'		Last 2 months		Change	Rat and sex	Before air blasting		Last 2 months		Change
	N¹	Average systolic pressure	N¹	Average systolic pressure			N¹	Average systolic pressure	N¹	Average systolic pressure	
		mm.Hg		*mm.Hg*	*mm.Hg*			*mm.Hg*		*mm.Hg*	*mm.Hg*
1 m	3	124	4	161	+37	18 f	3	108	4	169	+61
2 m	1	128	2	163	+35	19 f	1	105	5	161	+56
3 f	2	106	3	135	+29	20 f	3	117	3	168	+51
4 m	2	115	2	131	+16	21 f	2	99	4	150	+51
5 m	1	117	2	122	+5	22 m	2	108	3	149	+51
6 f	3	130	3	134	+4	23 f	2	104	4	154	+50
7 f	1	127	3	130	+3	24 m	2	98	4	146	+48
8 f	1	113	2	112	−1	25 m	2	123	3	170	+47
9 f	2	125	2	119	−6	26 m	2	116	4	161	+45
10 f	2	128	4	110	−18	27 m	2	111	3	154	+43
11 f	2	154	2	129	−25	28 m	1	107	1	148	+41
12 m			3	123		29 f	3	110	4	143	+33
13 m			2	136		30 f	2	139	5	171	+32
14 m			2	104		31 m	2	122	3	150	+28
15 m			2	122		32 m	3	105	4	130	+25
16 m			2	120		33 m	1	104	3	128	+24
17 f			3	114		34 m	2	132	3	155	+23
						35 f	2	117	3	137	+20
						36 f	2	118	5	126	+8
						37 m			4	160	
						38 m			4	151	
						39 f			4	170	
						40 f			4	183	
						41 f			5	170	
						42 f			3	141	
Mean....		124		127	+3	Mean..		113		154	+41

¹ Number of days on which blood pressure was determined.

RESULTS WITH THE PLETHYSMOGRAPH

Average blood pressures of experimental rats taken before air blasting was begun and of control rats during the same period are presented in table 1.   Since no sex difference of importance was noted, males and females are listed together.   During

the control period the mean systolic pressures of the two groups of rats were as follows: for experimentals, 113 mm.; for the controls, 124 mm. Hg. The higher average pressure for the latter group reflects the policy of including in it, whenever possible, rats with higher readings.
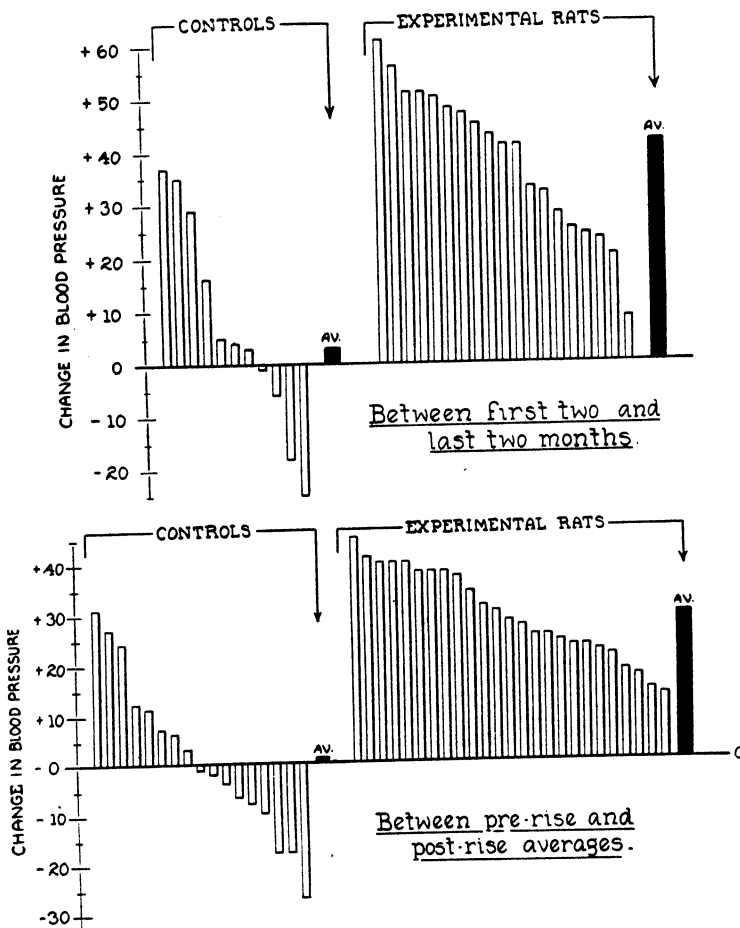
Fig. 1A (*top*). CHANGES IN BLOOD PRESSURE of controls and of experimental rats between first 2 and last 2 months. 1B (*bottom*). Changes in blood pressure of controls and of experimental rats between pre-rise and post-rise averages.

The average blood pressure of each rat in the last two months of the experiment is shown in the same table. Here the mean for the experimental animals was 154 mm. and for the controls, 127 mm. The mean rise over the control period was 41 and 3 mm., respectively, the former change being statistically highly significant (p < 0.01). Individual alterations in blood pressures are shown in figure 1A. In all of the air-blasted rats the change was in the direction of increase and in the controls was in both directions. The frequency distribution of the average systolic pressures of the rats in the last two months shows that measurements of 150 mm. or more were found in 16 (64%) of the experimental animals and in 2 (12%) of the controls (table 3).

Inspection of the protocols showed that the increase in blood pressure in the majority of the experimental animals occurred rather abruptly, taking place in the 8th to the 11th month after air blasting was begun. When the mean blood pressure per month in the experimental group was calculated, the sharp rise was seen to occur in the ninth month of auditory stimulation, increasing thereafter to a level of 150 mm. (fig. 2). The average monthly systolic pressure in the control group was at its highest level early in the experiment and did not exceed 133 mm. in any month.

Because of the restlessness of the animals during the determinations, readings were not obtained for 6 experimental and 6 control rats during the first two months (table 1). It was thought desirable to show the average blood pressures of these rats in the early stages of air blasting for comparison with later determinations and incidentally thereby to increase N (the number of days on which readings were ob
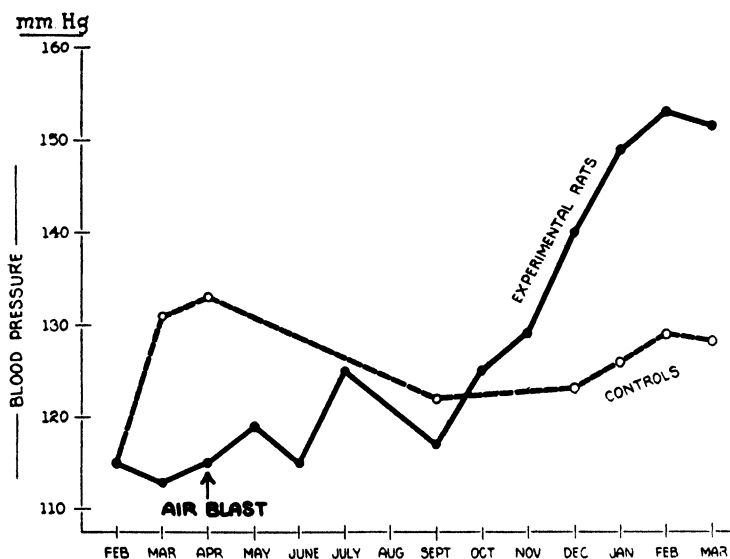
Fig. 2. AVERAGE MONTHLY BLOOD PRESSURES for one year of controls and of experimental rats

tained). Accordingly, for each control rat the readings in the 10th, 11th and 12th months were averaged, as well as the readings in the previous months. The mean systolic pressure and standard deviation of the controls for the last 3 months of the experiment was 125 ± 4 mm. and for the preceding period, 124 ± 2.6 mm. It may be seen that the figures differ only slightly from those given in table 1.

The readings of each air-blasted rat were scrutinized, and the measurement was noted which first indicated a rise in the animal's blood pressure. All measurements up to this one were averaged and all readings including and following it. The former are referred to in table 2 as pre-rise and the latter as post-rise averages. The individual changes are shown in figure 1B.

The mean post-rise blood pressure of the experimental group was 152 mm., an increase of 29 mm. over the pre-rise level of 123 mm. This change is statistically significant (p < 0.01). Twelve (48%) of these rats and 2 (12%) of the controls had blood pressures of 150 mm. or greater (table 3). With this treatment of the data it

TABLE 2.   AVERAGE SYSTOLIC BLOOD PRESSURES IN EXPERIMENTAL RATS BEFORE AND AFTER INITIAL RISE OF BLOOD PRESSURE

RAT AND SEX	PRE-RISE		POST-RISE		CHANGE
	N[1]	Average systolic pressure	N[1]	Average systolic pressure	
		mm.Hg		*mm.Hg*	*mm.Hg*
25 m	7	129	7	174	+45
20 f	9	117	9	158	+41
30 f	8	127	9	167	+40
18 f	8	130	7	170	+40
39 f	3	130	4	170	+40
26 m	7	116	12	154	+38
27 m	6	112	12	150	+38
40 f	5	141	5	179	+38
22 m	8	109	6	146	+37
21 f	11	121	3	155	+34
31 m	7	118	7	149	+31
37 m	5	139	7	169	+30
24 m	9	123	3	151	+28
34 m	7	128	3	155	+27
23 f	10	122	5	147	+25
38 m	3	123	9	148	+25
28 m	5	121	3	145	+24
42 f	8	125	2	148	+23
33 m	7	108	8	131	+23
32 m	7	118	8	140	+22
35 f	9	121	7	142	+21
19 f	7	129	7	147	+18
41 f	1	130	6	147	+17
29 f	10	115	6	129	+14
36 f	7	121	8	134	+13
Mean........		123		152	+29

[1] Number of days on which blood pressure was determined.

TABLE 3.   FREQUENCY DISTRIBUTION OF AVERAGE BLOOD PRESSURES

RANGE OF PRESSURES	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL
	Last 2 months of experiment		From 10th month	After initial rise in blood pressure
mm. Hg				
100–109	1	0	3	0
110–119	4	0	3	0
120–129	5	2	5	1
130–139	5	2	4	2
140–149	0	5	0	10
150–159	0	6	1	6
160–169	2	5	1	2
170–179	0	4	0	4
180–189	0	1	0	0

was to be expected that fewer experimental rats would be found to have readings over 150 mm. since the averages included early, relatively smaller increments of pressure. Nevertheless, the percentage of animals with elevated pressure in the experimental group is considerably higher than in the controls.

TABLE 4. BLOOD PRESSURES OBTAINED BY HAMILTON OPTICAL MANOMETER AND TAIL PLETHYSMOGRAPH

ANIMAL AND SEX	DIRECT (ETHER ANESTHESIA)		INDIRECT (UNANESTHETIZED)
	Systolic	Diastolic	Systolic blood pressure (Mean)
	mm. Hg	*mm. Hg*	*mm. Hg*
Controls			
1 m	175	88	161
11 f	158	67	129
12 m	155	88	124
14 m	152	95	106
8 f	146	83	103
10 f	135	85	110
5 m	130	78	123
15 m	128	80	122
9 f	115	75	119
6 f	105	60	135
3 f	98	58	135
Mean.	136	78	124
Experimentals			
37 m	163	110	169
33 m	162	98	128
22 m	160	100	146
35 f	156	107	139
36 f	155	100	134
25 m	155	82	174
20 f	150	95	158
26 m	138	72	151
18 f	135	90	170
30 f	135	75	171
38 m	134	90	148
23 f	130	75	147
29 f	112	63	143
Mean.	145	89	152

Direct Measurements. The data obtained with the optical manometer are shown in table 4. The average diastolic pressure of the control rats was 78 mm. Hg and of the experimental rats, 89 mm. Hg. The difference between the two groups is significant statistically ($P < 0.05$). Inspection of the individual values shows that diastolic pressures of 90 mm. or more were recorded for 8 of the 13 experimental rats but for only 1 of the 11 controls. Treatment of the data by the Chi-square method gave a probability of less than 0.01 that this relation was a chance one.

The average plethysmograph readings up to the date of the Hamilton measurements are also recorded, being the post-rise averages of the experimental rats and the averages of the controls covering the last three months of the experiment. The mean of the readings in the former group is significantly higher than that of the controls. The mean systolic pressure of the experimental rats recorded directly is also higher than that of the controls but not significantly so. The agreement is poor between the indirect and direct systolic readings in the same rat. (Coefficient of correlation is 0.26.) This was thought to be the result of the different circumstances under which the blood pressures were obtained.

TABLE 5. PLETHYSMOGRAPH MEASUREMENTS OF BLOOD PRESSURE DIRECTLY BEFORE AND AFTER HAMILTON MANOMETER DETERMINATIONS IN THE SAME RAT

RAT[1]	PLETHYSMOGRAPH PRECEDING HAMILTON[2] (B)	HAMILTON (LIGHT ETHER) (A)	(A–B)	PLETHYSMOGRAPH FOLLOWING HAMILTON (LIGHT ETHER)[2] (B')	(A–B')
	mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
1 m c	122	127/68	+5	116	+11
14 m c	126	152/95	+26	161	−9
12 m c	101	132/75	+31	135	−3
26 m e	137	115/66	−22	107	+8
8 f c	162	146/83	−16	140	+6
10 f c	121	135/85	+14	126	+9
3 f c		98/58		100	−8
6 f c		105/60		105	
35 f e		156/107		153	+3
29 f e		112/63		114	−2
37 m e		163/110		158	+5
38 m e		134/90		205	−71

[1] m = male; f = female; c = control; e = experimental.
[2] Each figure represents the average of at least 3 consecutive readings.

This impression was verified by taking indirect meaurements on a number of unanesthetized rats immediately before the Hamilton determinations and immediately after while the rats were still etherized. The data from the study are shown in table 5. It may be seen that there is poor agreement, on the whole, between the Hamilton values (*column A*) and the preceding plethysmograph results (*column B*) and that the differences are of the same order of magnitude as shown in table 4. The coefficient of correlation between *columns A* and *B* is low (0.36). There is, however, close agreement between the Hamilton and the following plethysmograph readings (*column B'*) in 11 of the 12 anesthetized rats, the difference being no greater than 11 mm.Hg in any individual animal. The coefficient of correlation here is 0.83.

ANATOMICAL FINDINGS

Examination of the ocular fundi in the living animals during the next to the last month of the experiment revealed no abnormalities[4]. At the close of the experiment

[4] We are indebted to Dr. Irving H. Leopold of the Hospital of the University of Pennsylvania for this information.

a number of the rats were killed, and various organs were weighed and preserved for histological examination.   No changes of significance in the microscopic appearance of the adrenal glands were found.   Although the average weight of the glands in the air-blasted rats exceeded the control values, the difference was not statistically significant.   The weights of the cardiac ventricles were not significantly different from the expected weights calculated by Rytand's formula (6).   While the average ventricular weight of experimental rats was heavier than that of control rats, statistical analysis showed that no significance may be attached to the difference.

<div align="center">DISCUSSION</div>

The results described above verify the findings of Medoff and Bongiovanni (1) that an elevation of systolic blood pressure may be found in rats subjected to prolonged, repeated auditory stimulation.   From unpublished data of their experiments we have ascertained that a nine month's exposure to the sound of the air blast intervened before the second (high) blood pressures were recorded.   Apparently, under the conditions of the experiments conducted by these authors and repeated by us, approximately that length of time must elapse before a change is noted in the levels of blood pressure.   It would be interesting to determine the effects of a longer daily exposure to the air blast or of an exposure oftener than once a day.   In spite of the fact that the results with the control rats seem to eliminate an age change as the explanation for the higher pressures in the experimental rats, it would be desirable to bring about the result in a shorter time if only for the sake of convenience.

Direct blood pressure determinations with a Hamilton monometer failed to corroborate our previous finding of a significant increase in the systolic blood pressures of the experimental rats.   However, when the blood pressure was recorded by the tail plethysmograph and the Hamilton manometer under as nearly identical conditions as was possible for us to obtain, the readings agreed quite closely.   This would be expected from reports in the literature comparing the two methods (4, 5).   These observations suggest that the procedures accompanying the direct method may have altered the blood pressure as found in the intact, unanesthetized rat.   Traumatization and etherization, the depth of which is difficult to control, may be factors that either raised or lowered the systolic pressure.   The question arises whether the diastolic pressures were not also changed by ether administration.   Studies on the effects of ether upon the circulation reveal that it causes widespread peripheral relaxation, which would only serve to lower the diastolic pressure (7).   Actually our data reveal a significant increase in diastolic pressure in the experimental group, as recorded upon etherized rats.   This is all the more impressive in face of the over-all lowering of the systolic pressure in these same animals apparently due to the ether anesthesia and other circumstances involved in the recording of direct blood pressures.

Systolic pressures of 150 mm. or greater in 64 per cent of the air-blasted rats exceed values for normal rats reported by various authors who have used the tail plethysmograph.   Readings no higher than 140 mm. in unanesthetized rats were recorded by Sulkin and Brizzee (8).   The figures of Williams, Wegria and Harrison (9) show that less than 5 per cent of 1207 animals had systolic pressures of 140 to 150 mm.   Loomis (10) reported 12 per cent of 100 rats with blood pressures of 140 to 150

mm. and 4 per cent between 150 and 160 mm. With other indirect methods similar results were obtained. In direct blood pressure measurements using Hamilton's optical manometer, Schroeder (11) found in 124 normal rats anesthetized with pentobarbital levels of 141 to 150 mm. in 11 animals and 151 mm. or more in 14 rats. That is, 11 per cent of his population had systolic blood pressures over 150 mm. The most stringent treatment of our data revealed an elevation of blood pressure over 150 mm. in 48 per cent of the rats.

While Grollman (12), Reed *et al.* (13), and others consider that a sustained average systolic blood pressure of 150 mm., as measured by the tail plethysmograph, indicates hypertension in the rat, we do not suggest that the elevation of blood pressure found in our experimental rats is comparable to that produced in renal hypertensive animals in which pressures of 180 mm. and higher are not uncommon and in which pathologic changes in the kidneys are demonstrable. Our experiments seem to show, however, that prolonged, repeated exposure to a stimulus that is of an exteroceptive (auditory) nature resulted in an increase in both systolic and diastolic blood pressure over the previous levels in the same rats and over the blood pressures of control rats.

SUMMARY

After a year's exposure to the sound of a blast of compressed air, 5 minutes a day for 5 days a week, the average systolic pressure of 25 gray Norway rats rose from an initial value of 113 mm. to 154 mm. in the last 2 months. The change is statistically highly significant. A control group of 17 animals, most of them litter mates of the experimental rats, showed an increase from 124 to 127 mm.Hg in the same period. Sixteen of the air-blasted animals (64%) and 2 of the controls (12%) had average blood pressures of 150 mm. or more at the close of the experiment.

Monthly averages indicated that the rise in systolic blood pressure of the experimental rats tended to occur in the ninth month after air blasting was begun. In the majority the increase occurred rather abruptly. The average of all systolic pressure readings in each rat before and after the rise showed an average change of + 29 mm. The difference is statistically highly significant. No such change was observed in the control group.

A significant increase in diastolic pressure (recorded under ether anesthesia with a Hamilton manometer) was found in a number of the experimental rats. There was no correlation of direct systolic blood pressure readings taken under ether anesthesia and indirect readings taken on the same but unanesthetized rats. There was a good correlation of indirect and direct systolic measurements when taken under as nearly identical circumstances as possible.

The results of the experiment suggest that an auditory stimulus applied over a prolonged period of time may produce an increase in the systolic and diastolic pressure of the rat.

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ADRENALECTOMY AND BLOOD PRESSURE OF RATS SUBJECTED TO AUDITORY STIMULATION

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PREVIOUS investigations on the effect of prolonged auditory stimulation in rats indicated that an elevation of blood pressure resulted in the majority of the animals (1, 2). It seemed possible that the experimental procedure constituted a chronic alarming stimulus and produced the increase in blood pressure through the pituitary-adrenal cortex mechanism (3–5). As a step toward testing this hypothesis, rats with elevated blood pressures following prolonged air blasting and a group of control animals were subjected to adrenalectomy and subsequent replacement therapy. If the elevation in blood pressure was mediated by the adrenal cortex, the blood pressures of both groups would be expected to return only to normal on replacement therapy. If, on the other hand, adrenal cortical overactivity was not responsible for the elevation of blood pressure in the experimental animals, restoration therapy might be expected to produce some restoration of elevated blood pressure in the air-blasted animals. In support of this concept, some restoration of hypertension due to renal ischemia (7–15) and intracisternal kaolin (16) does occur.

METHOD

Seven experimental and 4 control rats, ranging in age from $1\frac{1}{2}$ to 2 years, were chosen from the groups already reported (2). The former, subjected to auditory stimulation for a year, had exhibited a sustained elevation of systolic blood pressure during the last 2 to 4 months of air blasting. The blood pressure of 3 of the controls had remained unchanged during the year, while the fourth rat had developed hypertension spontaneously. Blood pressures were taken with a tail plethysmograph without anesthesia (2). The 11 rats were adrenalectomized under ether anesthesia through the mid-dorsal approach and given 10.0 cc. of physiological saline solution subcutaneously following operation. They were maintained thereafter on one per cent sodium chloride in the drinking water. Exposure of the experimental rats to the air blasting was continued to eliminate the possibility of a fall in blood pressure due to cessation of the auditory stimulus. A prompt fall in blood pressure occurred after adrenalectomy. The blood pressures were followed for an average period of four weeks, during which time it was ascertained that a pronounced fall had occurred. Adrenal cortical extract[1] was then administered intramuscularly to both groups in a dosage of 1.0 cc. per animal per day for 12 consecutive days. During the time be-

tween adrenalectomy and the beginning of cortical extract therapy, small doses (2.5 mg.) of DCA[2] were given to prevent collapse from acute cortical insufficiency, the maximum for any one rat being 10.0 mg. of DCA over a period of more than two weeks.   Blood pressure determinations were made every other day during cortical extract administration.

TABLE 1.   AVERAGE SYSTOLIC BLOOD PRESSURES OF AIR-BLASTED AND CONTROL RATS BEFORE AND AFTER ADRENALECTOMY AND REPLACEMENT THERAPY

	AVERAGE SYSTOLIC BLOOD PRESSURE IN MM. Hg			
NO. OF RAT AND SEX	Before adrenalectomy		After adrenalectomy	
	(1) Initial	(2) After 1 year's observation	(3) Salt only	(4) Stabilized on salt & cortin
Air Blasted				
20 f.	117	168	117	126
22 m.	109	149	110	131
25 m.	129	170	125	119
35 f.	121	137	98	123
37 m.	139	160	92	120
39 f.	130	170	104	121
40 f.	141	183	109	137
Mean.........	127	162	108	125
Control				
1 m.	120	161[1]	111	160[1]
10 f.	117	110	77	95
12 m.	131	123	100	122
13 m.	131	136	99	118
Mean.........	125	125	97	112

[1] This value not included in computing mean or statistical significance.

RESULTS

The results of the experiment are shown in table 1.   The blood pressure determinations presented before adrenalectomy are averages obtained before and after the elevation in blood pressure had occurred, as explained in the previous paper (2).   The average initial blood pressures of the experimental and control animals were 127 and 125 mm. Hg, respectively (column 1); during the last two momths of the year's observation the averages of the air-blasted and the control rats were 162 and 123 mm., respectively (column 2), not including the one control showing a spontaneous hypertension of 161 mm.   Following adrenalectomy the blood pressures of both groups fell within a week to subnormal levels; i.e., 108 and 97 mm. Hg (column 3).   The blood pressures did not reach lower levels because of the small doses of DCA admin-

[2] 'Cortate' kindly furnished by Schering Corporation.

istered at appropriate intervals. After the administration of cortical extract the blood pressures of both groups rose in seven days or less to 125 and 112 mm. (column 4), except for the spontaneously hypertensive control, whose pressure returned to the pre-adrenalectomy value. These levels were maintained for a minimum of five days before the experiment was terminated.

The experimental data were subjected to statistical analysis. The fall in blood pressure following adrenalectomy and the subsequent rise after treatment with adrenal cortical extract were significant both in the experimental ($p < 0.001$; $p < 0.05$) and in the control rats ($p < 0.02$; $p < 0.01$). The blood pressure of the experimental animals after restoration therapy was significantly lower ($p < 0.001$) than the pre-adrenalectomy figure, while there was no significant difference between comparable blood pressures in the controls. The blood pressures of both groups on cortical extract did not differ significantly from the initial values. There was no statistically significant difference between the blood pressures of the experimental and control groups when given replacement therapy after adrenalectomy.

<div align="center">DISCUSSION</div>

From the foregoing data it may be seen that in no instance was there any restoration of elevated blood pressure in the adrenalectomized animals except in the case of the spontaneously hypertensive control, where restoration was complete. In both the controls and air-blasted rats, cortical extract did produce a rise in blood pressure to normal levels.

It has been shown repeatedly that after adrenalectomy cortical extract is capable of partially restoring hypertension due to renal ischemia (7–15) and intracisternal kaolin (16). Two reports were found in the literature (17, 18) giving relatively negative results. In none of the papers in which the dose of cortical extract was specified did the dose exceed that used here.

Since comparable doses of adrenal cortical extract produced to a degree restoration of renal hypertension after adrenalectomy and only returned the blood pressure of our animals to normal, it is suggested that adrenal cortical overactivity may play some essential rôle in audiogenic hypertension. The elevated blood pressure of the spontaneously hypertensive control apparently did not depend on this mechanism.

In experiments by Selye (5) hypertension and nephrosclerosis similar to that produced by DCA overdosage have resulted from the exposure of rats to chronic alarming stimuli; i.e., cold, formalin and exercise. Auditory stimulation is not known to be a physically damaging agent, and the exercise with occasional seizures resulting from the excitement did not seem to be of the same magnitude as that used by Selye. Also high salt intake was necessary to sensitize his animals to the effects of exercise. Thus it seems improbable that exercise was the alarming stimulus in our experiments, but fear has been considered a potent alarming stimulus (4) and may have been the initiating factor here.

The adrenal medulla has not been ruled out as a possible source of the elevated blood pressure in these animals. Because of the difference in response of these animals from those with renal ischemia, it is likely that the kidney is not primarily involved in this type of experimental hypertension, but the possibility of secondary renal involvement as suggested by Selye and Stone (6) must be considered.

SUMMARY

To investigate the possible rôle of the adrenal cortex in the production of elevated blood pressure after prolonged auditory stimulation, adrenalectomy, with subsequent restoration therapy using adrenal cortical extract and sodium chloride, was performed on a group of experimental and control animals. Adrenalectomy lowered the blood pressure of both groups to subnormal levels. Replacement therapy with cortical extract and salt resulted in a return to normal systolic pressure in both groups. Hypertension was restored only in the case of a spontaneously hypertensive control. It is suggested that the adrenal cortex mediated the elevation of blood pressure occurring during prolonged auditory stimulation.

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ASYSTOLIC ARTERIAL PRESSURE GRADIENT AS A MEASURE OF LOCAL PERIPHERAL RESISTANCE[1]

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PERIPHERAL resistance may be defined as the frictional force opposing blood flow from the arterial to the venous system. The total force is composed of several resistances offered by various sizes of vessels, i.e., arteries, arterioles, capillaries and veins. Of these, the principal resistance results from the draining arterioles and is a function of blood viscosity and arteriolar diameter. There are a number of regional shunt pathways between the arterial and venous system, which are arranged in parallel and are related to total peripheral resistance (TPR) by the equation (1, 2): $\frac{1}{TPR} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3}$, etc., in which R_1, R_2, R_3, etc., refer to resistances of local circulations in various organs and areas of the body.

It is well known that while peripheral resistance may change equally throughout the body, territorial distribution of resistance may vary without altering TPR. It therefore seemed important to attempt to measure the relative resistances of these local circulations, in order to evaluate the part each might play in the total peripheral resistance. Of special interest were the changes which might occur in local areas under the influence of vasoactive drugs, temporary cessation of blood flow and altered physiological states such as hypertension and shock.

We have defined the asystolic arterial pressure gradient as the descending curve of intra-arterial pressure fall following sudden occlusion of a major artery supplying a local circulation. This report concerns an evaluation of the validity of the use of the gradient as a measure of local peripheral resistance; subsequent reports will deal with methods of measurement of the gradient and its use in altered circulatory states.

METHOD

Twenty-five dogs have been used and several hundred curves were obtained. The dogs were anesthetized with nembutal (0.02 gm/kg. intravenously) followed by smaller doses given as necessary to maintain anesthesia. Arteries supplying single circulations were chosen, i.e., the brachial, femoral, superior mesenteric and renal, in order to provide major portions of the circulation for measurement. Of other major areas, the carotid was not used because of the extensive collateral circulation to the brain in the dog. A 22- or 24-gauge needle connected to a Hamilton optical manometer was inserted into the lumen of the artery chosen, directed distally, and the artery occluded at a point proximal to the needle. When the renal, femoral and brachial vessels were used, this point was usually within 3 cm. of the needle; in the case of the superior mesenteric, the occlusion was made near its origin and the

needle, directed proximally, tied into a small branch close to the intestine. The occlusions were made to coincide as nearly as possible with the peak of systole. The subsequent intra-arterial pressure changes, distal to the point of occlusion, were recorded on a photokymograph for about 8 to 12 seconds, and the occlusion then removed. Occlusions were sometimes accomplished by digital pressure or ligature but a rubber padded hemostat was found to be more satisfactory. To avoid mechanical artifacts, it was very important that changes in the relative positions of the artery to the needle be prevented during the gradient determination, and that the artery be as well exposed for the occlusion as experimental conditions permitted. A period of no less than 15 seconds between one occlusion and the next was found to be advisable. Systemic blood pressure was sometimes measured simultaneously from another artery with a second Hamilton manometer.

Measurements in unexposed arteries were made in a few dogs before and after anesthesia was induced. They were also made in several human subjects, using the unexposed brachial or radial artery, and occluding the arterial supply to the arm by means of a pneumatic cuff inflated rapidly from a large reservoir.

In 6 experiments gradient determinations were correlated with simultaneous measurements of femoral or brachial blood flow. The arteries were cannulated and blood flow measured by a recording rotameter (3) which was placed in a circuit between one femoral artery and the other or between the carotid and brachial arteries. Gradients were measured with the 'arterial needle' placed in the connecting rubber tubing as close to the cannula as possible with occlusion just proximal to this point. Simultaneous measurements of systemic blood pressure were made with another Hamilton manometer from the carotid or femoral artery. As an anticoagulant pontamine fast pink (type DL—Dupont) combined with heparin in normal saline was injected slowly by vein. The initial dose was 150 mg/kg. and 1 mg/kg., respectively, with subsequent hourly sustaining doses of 100 mg. pontamine plus 1 mg. heparin. In these and in other experiments, one or two wire tourniquets were placed about the limb, near the axilla or in the mid-thigh region, beneath the respective artery, ve. 1 and adjacent large nerves, in order to occlude collateral circulation.

All records were measured, at least twice, in a specially constructed grid in which the pressure was determined at accurate eighth-inch intervals. As different photokymographs and film speeds were used, this permitted measurements to be made every 0.12 to 0.7 second and linear plots of the curves to be constructed. The accuracy of the method was found to be ± 2 mm. Hg on repeated measurements.

The gradients were measured in the following manner: the mean blood pressure of the cardiac cycle immediately preceding that of the gradient was used as the initial pressure of each gradient (cf. below). The point at which a smooth descending curve began was used as the starting point from which measurements of pressure and post-occlusion time were made. Characteristics of the curve within the first 8 seconds after occlusion were usually studied. The methods of quantitating and expressing changes of resistance will be discussed in another report.

RESULTS

Figure 1 exemplifies a typical response of the femoral arterial gradient to the intravenous injection of 100 γ of epinephrine. Blood flow and systemic blood pressure were measured simultaneously. Mean blood pressure rose abruptly following epinephrine and during its decline femoral vasodilatation was indicated by increased blood flow and a lowering of the gradient. Later, when mean systemic blood pressure was almost the same, femoral vasoconstriction was shown by a decrease in femoral blood flow and a rise of the gradient. Figure 2 shows representative femoral gradient responses obtained before and after injection of epinephrine and later of sodium nitrite. No attempt was made to control collateral circulation in this experiment. These examples are illustrative of the responses obtained in most experiments. Thus it becomes evident that the asystolic arterial pressure gradient can be used as an index of changes in local peripheral resistance.

The greatest part of the pressure fall of the gradient (65–85%) occurred during the first 1.5 seconds. This portion was arbitrarily designated as the α section. The second portion declined more gradually and was defined as the β section of the gradient. Regional α and β differences appeared in the records. The renal and mesenteric arterial gradients showed an early rapid fall of pressure in contrast to a more gradual decline in the femoral and brachial arteries (fig. 3). In each instance, however, the pressure fall continued in all determinations for as long a period as we have measured (2 minutes).

DISCUSSION

The diastolic slope of arterial pressure has long been known to reflect changes of total peripheral resistance, being diminished when resistance is high and increased when it is low (1, 4). Following arterial occlusion the subsequent intra-arterial hemodynamics depend on several factors. The propulsive force acting to empty blood from the arterial segment is at first chiefly dependent upon the pressure prevalent at the instant of occlusion and then is sustained by the elastic recoil of the arterial wall. The force resisting draining is due principally to the frictional resistance of the arterioles imposed upon the outflowing blood. Both arteriolar diameter and blood viscosity interact to produce this resistance. Thus, with the inflow cut off, the blood volume within the arterial segment is steadily diminishing during a gradient determination. The asystolic pressure gradient delineates two pressure flow curves: the first, or α gradient, under relatively physiological conditions, and the second, or β gradient, with a prolonged period of little flow driven by a lower pressure head at a decreasing velocity and increasing effective viscosity. At any given instant, therefore, the intra-arterial pressure and blood volume are the net results of all these forces. The influences which act to produce the shape of the curves obtained in these experiments will be considered separately.

Intra-arterial Pressure at Instant of Occlusion. Arterial occlusions were timed to coincide as nearly as possible with the systolic peak, but were often made on the systolic or diastolic slope. Sometimes arterial compression itself altered the initial pressure from which the gradient dropped, adding variation to that already inherent in the timing of the occlusion. On study of successive curves, however, it soon became apparent that the initial pressure was less significant than was the mean pressure at the start of the gradient. A large difference in initial pressure between two curves with the same mean pressure might shift the timing of the gradient by as much as 0.10 second but the rates of fall of pressure and the shapes of the curves were essentially the same in each case.

Effect of Arterial Elasticity. Once systemic blood pressure is cut off, intraarterial pressure is directly related to the force of the elastic recoil of the arterial wall acting against the resistance of the arterioles. It is evident, therefore, that the gradient is a measure of both elasticity and resistance.

The directional changes of both arterial elastic recoil and arteriolar diameter are opposite to each other under most physiological conditions, and it is difficult to decide where the influence of one leaves off and of the other begins. Although we have not measured arterial elasticity, we believe that there is sufficient evidence to warrant

the conception that it plays a relatively minor rôle in the shape of the gradient. *a)* When the force of elastic recoil was increased by an increase in systemic blood pressure, the gradient should have shown a more rapid fall if the principal effect was due to elasticity. This did not occur. *b)* When the coefficient of elasticity was altered by drugs, the changes found were in the opposite direction to those expected if elas-
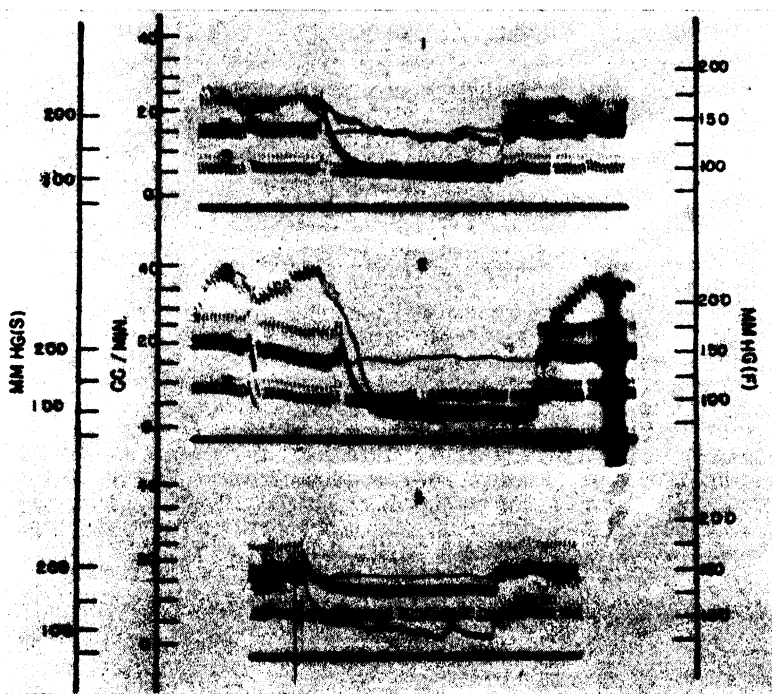
Fig. 1. SIMULTANEOUS DETERMINATIONS OF FEMORAL ARTERIAL GRADIENT AND FEMORAL BLOOD FLOW. *B* is the blood flow, measured with a recording rotameter, *F* the blood pressure of the left femoral artery, and *S* the systemic blood pressure measured in the right femoral artery. Time is indicated by interruptions of the *S* curve at 6-sec. intervals. A tight wire tourniquet was placed about the limb, occluding collateral circulation. The *S* systolic pressure has been marked manually, in order to intensify it. *Curve 1* was obtained 48 sec. before the intravenous injection of 100 γ of epinephrine, and *curves 2* and *3*, 25 and 143 sec. respectively, afterwards. Vasodilatation is shown in 2 by a lowering of the gradient, an increase of *B* accompanied by an increase of *S*. Vasoconstriction is shown in 3 by a rise of the gradient, and a decrease of *B*, without change in *S* from *curve 2*. Due to the hydrodynamics of the rotameter, flow is not shown as falling to zero during the period of occlusion. (*Dog W 25*—wt. 22 lbs.)

ticity were a major factor (figs. 1–3). *c)* A femoral arterial pressure-volume curve was constructed from published data (13) shown in table 1. Analysis indicates that arterial elasticity changes relatively little at the higher pressures (150–200 mm. Hg) when elastic limits are approached, but that the slope of the curve is essentially a straight line at pressures below 140 mm. Hg. At these lower pressures the rate of change of elasticity can be assumed to be almost constant. It is with these levels of pressure that the gradient is principally concerned.

Intra-arterial Blood Volume. It is probable that alterations of arterial elasticity
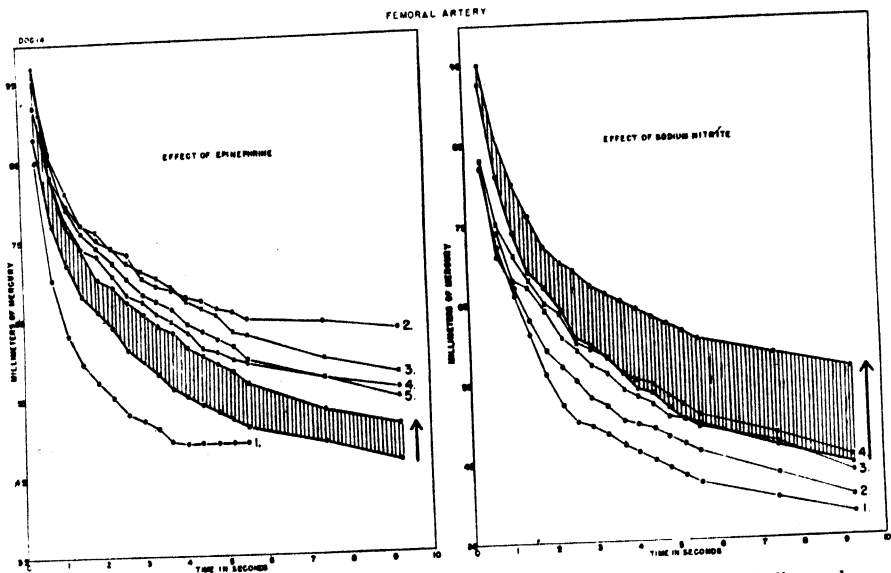
Fig. 2. REPRESENTATIVE FEMORAL GRADIENT RESPONSES. The shaded areas indicate the range of determinations of gradients (7 successive ones in A, and 4 in B) obtained at 12-sec. intervals during control periods. The arrow indicates the direction of change, the lower border being the first curve and the upper the last. Thus it is probable that vasoconstriction occurred although no attempt was made to control collateral circulation. A shows curves, numbered 1–5, obtained 30, 56, 81, 105 and 121 seconds after the intravenous injection of 100 γ of epinephrine. It would appear that a vaso-dilatation occurred, synchronous with the rise of systemic blood pressure, followed shortly by a progressively diminishing vasoconstriction while the pressure remained elevated. B shows curves, numbered 1–4, obtained 59, 79, 97 and 110 seconds, respectively, following the intravenous injection of 0.1 gm. sodium nitrite. An initial vasodilatation occurred which progressively diminished while systemic pressure fell continuously. (Dog W 14—wt. 30 lbs.)
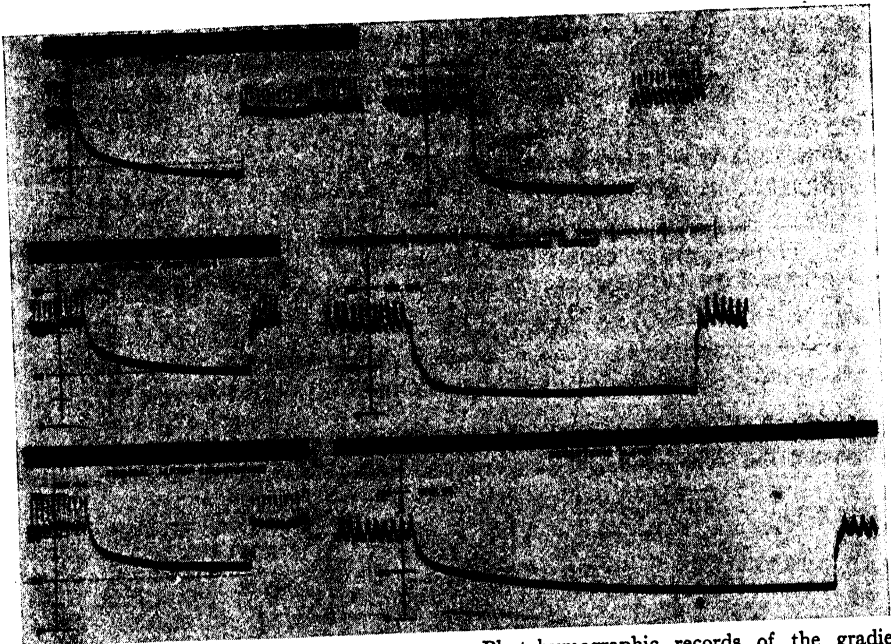
Fig. 3. RECORDS OF REGIONAL GRADIENTS. Photokymographic records of the gradients obtained from different arteries. On the left are curves obtained from the femoral artery (dog W 14) under various conditions, the middle being the control. The lower curve was made 56 sec. after the

do not cause great variations of femoral intra-arterial blood volume (table 1). The pressure-volume curve therefore can be used as an approximate index of blood flow under the conditions of gradient determination. Assuming that the pressure is related to the fluid content of the system at a specific instant, then because there is no inflow, the change of pressure during a given time is a measure of blood outflow.

Blood Viscosity. Blood viscosity has been assumed to remain constant under the experimental conditions. Presumably the effect of viscosity increases as flow decreases during prolonged arterial occlusion. The action of epinephrine in increasing hemoconcentration, and therefore viscosity, may influence our results, but this influence is probably small and may be absent when other drugs are used.

Repeated Determinations. Repeated gradient determinations, i.e., arterial occlusions, appeared to cause a slight degree of femoral, brachial and mesenteric vasoconstriction if performed at too short intervals, that is, less than 15 seconds apart (fig. 2). These were quickly reversible. If arterial occlusions were greatly prolonged marked vasodilatation occurred, presumably from reactive hyperemia. Reversible renal vasodilatation was generally found after rapidly repeated determinations. Dicrotic notches were not seen in any of the curves, even when marked vasospasm was induced.

Collateral Circulation. The contour and rate of pressure fall of the asystolic arterial pressure gradient may be markedly influenced by collateral circulation, except possibly in the kidney. Under normal conditions collaterals do not supply blood to these cognate[2] vascular beds as there is no pressure differential between the collateral and cognate systems. Intra-arterial pressure (P_A) falls in the cognate system during the gradient determination while the pressure within the collaterals (P_C) remains unchanged. Therefore, the differential pressure, $P_C - P_A$, increases proportionately to the fall of P_A. This differential pressure head and content of blood within the collaterals is transmitted into the cognate system in amounts proportional to the diameter of the collaterals and the magnitude of $P_C - P_A$. The amount of this blood flow may be considerable (5) and may increase with repeated gradient determinations.

The presence of collateral circulation is detected by *a*) pulsations in the curve synchronous with the pulse rate, *b*) a rise of pressure during the gradient determination and *c*) an increase of gradient pressure levels in sequential curves of a control period. Despite these facts it seems probable that the asystolic gradient is a measure of local peripheral resistance when the curve shows a smooth progressive non-pulsatile fall. There are several lines of evidence for this view. *a*) Gradient changes were found following the injection of a vasoactive drug in the absence of any change in systemic blood pressure. *b*) Gradient changes have been shown to occur in a direc-

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[2] Cognate denotes the direct lineal arterial pathways as contrasted to collateral pathways (5).

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intravenous injection of 100 γ of epinephrine, and the upper made 7 min. later. Note that the change in systemic blood pressure, indicated on the scale at the left, was relatively less than the change in the shape of the gradient. The curves obtained from the renal (upper right—*dog W 13*) and mesenteric arteries (middle right—*dog W 19*) show a more rapid fall during the α gradient period than do those from the femoral. The lower right curve (*dog W 19*) was made about 2 hours after the middle one, when the animal was in a state of incipient shock. Note that the shape has changed considerably and that the systemic blood pressure is lower. Time is indicated at 2-sec. intervals along the abscissa. No attempt was made to control collateral circulation in these experiments.

tion opposite to the change of systemic blood pressure (figs. 1 and 2A), which is contrary to what might be expected from the $P_C - P_A$ differential. *c*) Similar curves have been obtained following the action of a drug whether or not collateral circulation has been prevented (compare figs. 1 and 2A). *d*) In some instances, when collaterals were obvious, typical gradient changes occurred following the action of vasoactive drugs. *e*) Presumably the diameter of the collateral channels are influenced similarly to the arterioles by vasoactive drugs.

In two experiments (nos. *W-29* and *W-30*), with conspicuous collateral blood flow into the cognate bed, progressive ligation of all visible collaterals of the widely exposed femoral artery was accomplished, the process being interspersed with gradient determinations. Later wire tourniquets were applied and a comparison made of the

TABLE 1. CALCULATION OF THE VOLUME OF BLOOD IN THE FEMORAL ARTERY OF A 15-KG. DOG (0.5 SQ. M. BODY SURFACE)

PRESSURE	CC. BLOOD VOL. IN BOTH LEGS / SQ. M. BODY SURFACE	CALCULATED CC. BLOOD VOL. IN FEMORAL ARTERY
0	46.3	8.68
20	48.0	9.00
40	50.3	9.43
60	52.5	9.84
80	55.0	10.31
100	56.8	10.65
120	58.6	10.99
140	60.3	11.30
160	61.5	11.53
180	62.3	11.68
200	62.9	11.82

Data calculated from table 2 of the paper by Remington and Hamilton (13). The above values are probably high for femoral blood volume as pelvic blood supply was included in the original calculations. The femoral volume was considered to be three fourths of the total of that of a single hind leg.

gradients taken under the two conditions. The gradient fell more abruptly and the β section was at lower pressure levels after the tourniquets had been tightened.

There are no absolute criteria of vasoactivity in the determination of the gradient when collateral circulation is present, unless simultaneous measurements of blood flow are made. The only conditions under which one can be certain that collaterals have no effect upon the gradient are when flow is diminished, pressure unchanged or lower, and the gradient rises, a combination of changes indicating local vasoconstriction. Similarly, when flow increases, pressure is unchanged or rises and the gradient falls, vasodilatation without collateral interference can be assured. Thus, for example, a rise of the gradient following a drug injection, when collateral circulation is not excluded, may be due to pressure and flow transmission from the collateral into the cognate system and/or vasoconstriction. However, for the above reasons we believe that the asystolic arterial pressure gradient is a qualitative measure of local peripheral resistance in the presence of collateral circulation, and a quantitative measure if collateral circulation is excluded, provided that mean pressure is taken into consideration (6).

Effects of Prolonged Arterial Occlusion. In some femoral and brachial gradient determinations, arterial occlusion was prolonged up to two minutes. When the initial mean blood pressure was about 100 mm. Hg, intra-arterial pressure reached venous pressure levels in about two minutes when collateral circulation was excluded. With higher initial pressures, venous pressure levels were not reached during this time. Even with collateral circulation present, gradient pressures continued to fall at the end of two minutes. Such prolonged occlusions are followed by marked vasodilatation and increase of blood flow. It is doubtful, therefore, that any change of venous pressure with such drugs as epinephrine or sodium nitrite plays a part in the form of the gradient, for such changes are extremely small (7, 8).

The vasodilatation noted after prolonged arterial occlusion, in response to anoxia or the accumulation of metabolites, may occur at some point along the course of the gradient. We have seen no evidence, however, of sudden changes in the gradual decline of the curve, which would indicate a more rapid outflow of the blood captured in the local arterial network. In two experiments in man, using photo-electric plethysmographs on the fingers, prolonged brachial occlusions were followed by relatively rapid but small decreases in finger volume occurring 25 seconds later.

Finally, a word may be said about the levels of pressure at which flow ceases. Some investigators (9, 13), using data derived from perfusion of limbs (10), have considered that effective flow is proportional to pressure minus 20 mm. Hg, which suggests that below this level flow ceases. Our experiments do not support this hypothesis. Often gradients of the renal and mesenteric systems fell to 10 mm. Hg or lower in the first 3 seconds. In a few cases femoral and brachial arterial gradients affected by vasodilating drugs fell to a range of 12 to 17 mm. Hg within 10 seconds. During prolonged arterial occlusion pressure continued to fall as long as the artery was clamped. The rôle of dilatation induced by ischemia on the continued fall of pressure is not known, but if blood flow continues as long as pressure is falling, we believe that 20 mm. Hg is not the level of pressure at which flow ceases. Therefore, effective flow should probably be considered as proportional to arterial pressure minus venous pressure. The data of others (11, 12) bears this out.

SUMMARY

The asystolic arterial pressure gradient has been defined as the descending curve of intra-arterial pressure following sudden occlusion of a major artery. Evidence was presented that the gradient can be used as a measure of changes in the peripheral resistance of local areas of the circulation. This measure is strictly quantitative only if collateral circulation is excluded but it may be used as a qualitative index of resistance when some collateral circulation is present.

The hemodynamic factors involved in the formation of the contour of the gradient were discussed.

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INVESTIGATIONS OF FREE AND BOUND POTASSIUM IN RAT BRAIN AND MUSCLE[1]

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I T IS the purpose of the present paper to investigate what fraction of the total cellular potassium occurring in rat brain and muscle is diffusible. The existence of a non-diffusible form of potassium has been suggested by several investigators. Yannet (8) discusses a potassium fraction within the cell which is not osmotically active and refers to this as a 'bound fraction'. Fenn (2), in a review of the rôle of potassium in physiological processes, cites Ernst's conclusion that stimulation of a muscle results in the breakdown of 'potassium compounds' with a resulting loss of diffusible potassium from the cell, when cell permeability is increased, and later he refers to Neuschlosz's finding that a certain fraction of muscle potassium cannot be extracted from minced muscle by isotonic sodium chloride. Pichler (5) describes a water soluble, a lipoid soluble and a third potassium fraction found in the central nervous system of frogs and Reginster (6) reports the existence of two potassium fractions, a diffusible and a non-diffusible fraction, in muscle, the former increasing with activity. Several authors discuss the nature of bound potassium. Christiansen and Hastings (1) find that with increasing pH, cephalins bind appreciable amounts of potassium and Folch (3) claims that three brain lipids—cerebron sulfuric acid, phosphatidyl serine and diphosphoinositide taken together can bind 27 per cent of the potassium occurring in brain.

PROCEDURE

Ultrafilters. In the experiments that follow, an ultrafiltration apparatus was devised wherein collodion was used as a semipermeable membrane. To construct ultrafilters, visking cellulose sausage casing (20/23″ inflated diameter) was cut into six-inch strips and washed until sodium and potassium free. Strips were knotted at one end and tested for leakage, then passed over No. 2 rubber stoppers which had been fitted on separatory funnels, and bound tightly in place with washed No.

12 Cuddyhunk. The diameter of the Cuddyhunk is important; if too small, tight binding will tear the collodion; if too large, the binding will not be secure.

Linen or down-proofing bags which had been boiled and washed were secured around the collodion bags to prevent their exploding under pressure and the filters thus constructed were fitted into side-arm suction flasks and tested for leakage with distilled water under one atmosphere air pressure. To prevent leakage it is essential that the collodion be kept moist after once being wet. Liquid collodion was used to make air-tight seals between glass and rubber connections.

Ultrafiltration of control solutions. Control solutions containing known concentrations of sodium and potassium chloride were ultrafiltered for 12 to 18 hours under one atmosphere air pressure at 3°C. The ratio of the concentration of sodium and potassium cations passing through the filter to the concentrations of cations in the control solutions was determined by the Perkin-Elmer flame photometer (model No. 18).

Ultrafiltration of tissue homogenates. Male white rats weighing ca. 200 gm. were killed by ether and the brain (sans cerebellum) and left gastrocnemius muscle were quickly removed, weighed wet and each then mashed for four minutes in the Waring blendor in 100 or 200 cc. of cold distilled water. Mashing was carried out at 3°C.

Homogenates of brain and of muscle prepared in this manner were divided into three portions. Two of these were ultrafiltered under the same conditions as were the control solutions (see above); the third portion was stored 12 to 18 hours at 3°C. Potassium determinations were made on all three solutions by the flame photometer for a comparison between the filtered and non-filtered aliquots. Usually a further dilution to 1/300 was necessary for bringing readings into the range of our calibration curve of the flame photometer.

Whereas brain homogenates could be analyzed directly, the estimations of potassium concentrations in the non-filtered muscle solution were thought to be inaccurate, for muscle homogenates prepared on the Waring blendor characteristically contained visible strands of connective tissue. It was suspected that the photometer might not burn these strands completely and thus give low readings. As a check, one series of muscles was ashed in a muffle furnace at 550°C. for one hour, then taken up in 0.1N (potassium and sodium-free) hydrochloric acid and made up to 100 cc. volume with distilled water, while a second series of muscles was homogenized as above then boiled under a reflux condenser to prevent concentration of solutions, until the strands were broken up.

Comparisons of potassium concentrations were subsequently made between the muscle homogenates prepared by these three methods.

Ultrafiltration boiled tissue solutions. A less cumbersome method was devised wherein homogenization was not necessary. For determinations of the total amount of potassium contained in a tissue, we have found that an intact brain or muscle may be boiled under a reflux condenser in 100 or 200 cc. of distilled water for 10 minutes, then stored for 36 hours at 3°C. (7). At the end of this time ultrafiltrations of the liquid surrounding the fragmented tissue were carried out and potassium concentrations were determined simultaneously in both filtered and non-filtered aliquots of this fluid.

Refiltration of filtrates.   As controls of the method the filtrates of several homogenates were aliquoted and passed through a second ultrafilter.   Conditions of the second filtration were identical with those of the first.

Sodium determinations.   Wherever possible, the sodium concentrations of filtered and non-filtered aliquots were made concurrently.   Sodium determinations were made at dilutions of 1/100.

<center>RESULTS</center>

Ultrafiltration of control solutions.   In all cases where control solutions containing known concentrations of sodium and potassium were ultrafiltered, the ratio: Concentration of cation in ultrafiltrate/Concentration of cation in reagent solution was close to unity.   Discrepancies which were reported fell within the 5 per cent experimental error which is characteristic of our flame photometer.

Ultrafiltration of tissue homogenates.   A mean value of 3.47 ± 0.09 mg. K/gm. wet brain was found in the nonfiltered aliquots of brain homogenates.   In the case of muscle, three different mean values were derived from non-filtered aliquots prepared by the three methods described above.   The highest of these was 3.87 ± .06

<center>TABLE I.   MEAN VALUES OF BRAIN AND MUSCLE POTASSIUM CONTENT MG/GM.</center>

	BRAIN	MUSCLE		
	Homogenized	Homogenized	Homogenized boiled	Ashed
	A	*B*	*C*	*D*
Mean values..........	3.47 ± .09	3.56 ± .07	3.87 ± .06	3.74 ± .04
No. of expts..........	12	12	11	8

Comparing *columns B* and *C*, *t* = 3.3, *P* = 0.01; differences significant.

mg. K/gm. wet muscle derived by boiling muscle homogenates.   These values are presented in table 1.

Application of Fisher's *t*-test shows that there is a statistically significant difference between the mean 'homogenized' and mean 'homogenized-boiled' muscle values, *P* being 0.01.

For subsequent calculations on the percentage of diffusible potassium occurring in muscle, this highest value, 3.87 ± .06 mg. K/gm. wet muscle, was used as a standard denominator.

Analysis of the ultrafiltered aliquots of brain and muscle showed that 75 per cent and 72 per cent respectively of the total potassium were diffusible through collodion.   In the case of brain this fraction was determined by comparing the concentration of potassium in the homogenized filtered aliquot to the concentration of potassium in the homogenized-boiled, non-filtered aliquot.   Table 2 summarizes these results.

A mean value of 3.42 ± .08 mg. K/gm. wet brain and 3.96 ± .12 mg. K/gm. wet muscle were found by analysis of non-filtered boiled tissue solutions.   These values do not vary significantly from total potassium determinations made on tissue homogenates as shown above.   Comparisons are made in table 3.

Ultrafiltered aliquots of boiled tissue solutions showed 69 per cent and 70 per cent of diffusible potassium in brain and muscle respectively.

Table 4 presents a comparison between the percentage of non-diffusible potassium in brain and muscle as determined by the two ultrafiltration methods described. Application of Fisher's *t*-test shows that there is no statistically significant difference

TABLE 2.   MEAN VALUES, TOTAL AND NON-DIFFUSIBLE POTASSIUM IN BRAIN AND MUSCLE

| | TOTAL MG. K/GM. HOMOG. | MG. K/GM. HOMOG. & ULTRAFILT. | % NON-DIFFUSIBLE | TOTAL MG. K/GM. HOMOG. BOILED | MG. K/GM. | |
					Homog. & ultrafilt.	Non-diffusible
	A	B	C	D	E	F
Mean values.......	3.47 ± .07	2.60	25 ± 1.74	3.87 ± .06	2.90	28 ± 2.84
No. of expts........	12	14		11	15	

TABLE 3.   MEAN VALUES OF BRAIN AND MUSCLE POTASSIUM CONCENTRATIONS AS DETERMINED BY HOMOGENIZING VS. REFLUX-BOILING WHOLE TISSUES

| | BRAIN | | MUSCLE | |
	Homogenized mg. K/gm.	Reflux-boiled mg. K/gm.	Homogenized-boiled mg. K/gm.	Reflux-boiled mg. K/gm.
	A	B	C	D
Means..............	3.47 ± .09	3.42 ± .08	3.87 ± .07	3.96 ± .12
No. of expts..........	12	23	11	17

Comparing columns A and B, t = 0.42, P = 0.5; not significant.
Comparing columns C and D, t = 0.763, P = 0.4; not significant.

TABLE 4.   MEAN VALUES, COMPARISON OF ULTRAFILTERED TISSUE HOMOGENATES VS. REFLUX-BOILED SOLUTIONS

| | BRAIN | | MUSCLE | |
	Homogenized % non-diffusible K	Reflux-boiled % non-diffusible K	Homogenized % non-diffusible K	Reflux-boiled % non-diffusible K
	A	B	C	D
Means..............	25 ± 1.74	31 ± 2.84	28 ± 1.56	33 ± 2.05
No. of expts..........	14	18	15	19

Comparing columns A and B, t = 1.72, P = 0.1, no significant difference.
Comparing columns C and D, t = 1.94, P = 0.05, no significant difference.

between the percentages of non-diffusible potassium as determined by these two methods in brain or in muscle.

Refiltration of filtrates.   The refiltration of several filtrates, in a total of six experiments, showed that a mean value of 92 per cent of the potassium by concentration in both brain and muscle would pass through a second ultrafilter.

Sodium determination.   In a total of 14 experiments both sodium and potassium concentrations of filtered and non-filtered aliquots were obtained. Within experimental error 100 per cent of both brain and muscle sodium were diffusible while

in this same series 75 per cent of brain potassium and 78 per cent of muscle potassium were diffusible.

DISCUSSION

The total potassium values of brain and muscle reported in the present paper, 3.47 ± .09 mg. K/gm. brain and 3.87 ± .06 mg. K/gm. muscle, are in agreement with values reported elsewhere in the literature (4), as well as with values obtained by a second method herein reported.

Two methods for determining the percentage of non-diffusible potassium have been demonstrated and values of 30 per cent obtained by ultrafiltration of tissue homogenates and by the ultrafiltration of solutions in which intact brain and muscle had been boiled, show no statistically significant variation from each other; further corroboration of this figure of 30 per cent is offered by Folch (3) who reports a 27.2 per cent bound K fraction in brain.

As controls on the ultrafiltration method, we have shown that control solutions of sodium and potassium chloride can be vacuum ultrafiltered with accurate cation concentration determinations; further, the refiltration of filtrates reported, indicates that the potassium fraction which we have considered as free, is really diffusible through collodion. Repeated filtration of the free fraction consistently yields the same results; 92 per cent of the free fraction can be refiltered. The 8 per cent of the diffusible potassium fraction which is lost in the refiltration process may be due to any of several factors, but elaboration of this phenomenon v ould require more detailed investigation. While the collodion membrane allows passage to only 70 per cent of the total potassium, 100 per cent of the sodium filters through, thus further substantiating the reality of the bound fraction of potassium.

The nature of the potassium binding is not understood. Presumably potassium ions are associated with large thermolabile molecules. Folch (3) has attributed this binding rôle in the brain to lipids.

SUMMARY

To determine the percentage of diffusible potassium occurring in rat brain and muscle, an ultrafiltration process was developed where visking cellulose sausage casing served as a semi-permeable membrane. Following satisfactory ultrafiltration of control solutions, homogenates of rat brain and muscle were separately ultrafiltered. The percentage of diffusible potassium, in the case of brain, was calculated by comparing the potassium concentration in the ultrafiltrate to that of the original homogenate. In the case of muscle, the potassium concentration of the ultrafiltrate was compared to that of the original homogenate boiled. Mean values of 3.47 ± .09 mg. K/gm. brain of which 25 ± 1.74 per cent is non-diffusible and 3.87 ± .06 mg. K/gm. muscle of which 28 ± 2.84 per cent is non-diffusible were obtained. Total potassium content values of 3.42 ± .08 mg. K/gm. brain of which 31 ± 2.84 per cent was bound and 3.96 ± .12 mg. K/gm. muscle of which 33 ± 2.05 per cent was bound were obtained by the analysis of supernatant liquids in which whole tissues had been boiled.

As controls of the method, first, the refiltration of several filtrates showed that

a mean value of 92 per cent by concentration of both brain and muscle potassium would pass through a second filter and, second, within experimental error, 100 per cent of both brain and muscle sodium were diffusible while in the same series 75 per cent of brain and 78 per cent of muscle potassium were diffusible.

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CYCLIC CHANGES IN THE RESPIRATORY CENTERS, REVEALED BY THE EFFECTS OF AFFERENT IMPULSES[1]

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IT IS the purpose of this paper to present evidence of changes in the respiratory centers occurring progressively during inspiration and expiration. The rhythmicity of natural respiration may be explained by assuming that inspiration stops when these progressive changes reach a certain critical level and starts again when another critical level is attained. An analysis of such alterations in the centers can therefore contribute to an understanding of normal respiratory mechanisms.

In experiments previously reported, it was found that in natural breathing the frequency of discharge over phrenic motoneurones was unaffected by impulses from pulmonary stretch receptors until the very end of inspiration; then impulses from these receptors caused a very rapid decline in frequency and finally a cessation of the motoneurone discharge (5). Since afferent impulses from pulm·nary stretch receptors are known to be discharged at progressively increasing frequency throughout inspiration (1), these findings suggest that there may be a sharp threshold for stopping inspiration, which the afferent impulses must exceed before a detectable effect is produced. Such a sharply-defined threshold for stopping inspiration was indeed revealed by Boyd and Maaske (2) by electrical stimulation of the vagus nerves.

This well-defined threshold for stopping inspiration should be a useful tool for exploring changes in the centers during the respiratory cycle, alteration of the chemical environment of the center and under other experimental conditions. Changes during the respiratory cycle were investigated in the following experiments. During the course of the investigation it was found that further information concerning the changes occurring in the centers could be obtained by combining the study of the threshold for stopping inspiration with an analysis of the delay in start of inspiration caused by brief volleys of afferent impulses during expiration.

The results obtained by these two procedures were in general found interpretable in terms of current concepts of the organization of the respiratory centers.

METHODS

Experiments were performed on cats anesthetized with Dial.

In order to record impulses discharged by motoneurones supplying the diaphragm, the upper root of the phrenic nerve was cut as far distally as possible without opening the thorax. The central end of the nerve was freed from the surrounding tissues and placed on recording electrodes connected

to a vacuum tube amplifier and cathode ray oscillograph. For most experiments the end of the nerve was split into several fine strands and a strand was selected for recording in which only one fiber became active during each inspiration.

Respiratory movements were recorded by means of a tambour pressed against the abdomen. Electric stimuli were applied to the superior laryngeal nerve. In order to permit precise timing of the stimuli, with respect to the phrenic nerve impulses, the stimulator was keyed by the impulses themselves through a connection from the output of the amplifier. When a switch in this circuit was closed during expiration, the first impulse in the next inspiratory discharge turned on an electronic delay circuit. At the end of the delay, which was adjustable, the stimulator was automatically started and delivered a controlled number of stimuli at any desired frequency and intensity. The stimuli passed to the nerve through an isolating transformer which served to minimize spread of current. After each application of stimuli, a number of inspirations were allowed to occur normally before further stimulation, in order to avoid complications due to persisting effects of the tests in the respiratory centers.

The superior laryngeal nerves were chosen for stimulation in these experiments because it was known that afferent impulses in these nerves have a strong inhibitory action on inspiration. Moreover, it was hoped that the superior laryngeal nerves might contain fewer kinds of afferent fibers than the vagus nerves, which are known to carry afferent impulses from at least four sets of receptors subserving different respiratory reflexes.

The functions of even the superior laryngeal nerves are not simple, however, for impulses in their afferent fibers can increase as well as decrease the activity of phrenic motoneurones. This is apparent, for example, in the records reproduced in figure 1. Relatively weak stimuli (*record B*) had an inhibitory action on inspiration, indicated by a reduction in the frequency and duration of the motoneurone discharge and by a delay of the subsequent inspiration. When the strength of stimulation was increased, however, instead of a further decrease in number of impulses, there was an increase (*record C*); impulses were actually discharged for a longer time and at a higher maximum frequency than in a normal inspiration. These stronger stimuli thus initiated afferent impulses causing an excitatory effect on phrenic motoneurones, as well as the inhibitory effect revealed by the delay of the next inspiration.

Observations such as these indicate that the superior laryngeal nerve fibers which excite inspiration are of a higher threshold than those which inhibit it. Therefore, in order to minimize complications due to simultaneous excitor and inhibitor effects, we have in some experiments employed the weakest stimuli which gave a sufficient degree of inhibition. This procedure, however, was not altogether satisfactory, since with weak stimuli the number of fibers stimulated could vary widely from time to time, due to changes in irritability. Fortunately, it proved possible to obtain relatively uncomplicated inhibitory effects from stronger stimuli provided the period of application was kept short. This is illustrated in figure 2, where 3 stimuli (*record B*) stopped the inspiratory discharge after only 2 impulses. This occurred in spite of the fact that these stimuli were strong enough to have not only an inhibitory, but also an excitant action, shown by prolongation of the activity when more shocks were applied in *record C*. In many of the experiments to be presented in this paper, the inhibitory effect was even stronger than in *record B* of figure 2, for the discharge often stopped after the first impulse. To accomplish this, higher frequencies of stimulation than those illustrated in the records of figures 1 and 2 were usually employed.

<div align="center">RESULTS</div>

<div align="center">*Effects of Afferent Impulses During Inspiration*</div>

Afferent impulses early in inspiration. Figure 3 shows an experiment in which various numbers of afferent impulses were sent in over one superior laryngeal nerve immediately after the first impulse discharged by a certain phrenic motoneurone. As few as 2 volleys of afferent impulses noticeably delayed the discharge of the second impulse in this motoneurone (*record B*). Progressively greater delays were caused as the number of afferent volleys was increased to 4 and to 9 (*record C* and *D*). After

this a remarkable change occurred when only one more was added to the number of afferent volleys. For with the change from 9 to 10, the interval between impulses was increased more than five-fold (*record E*). Compared to this large change, additional stimulation had but little additional effect (*F*).

The results of a number of similar experiments are shown graphically in figure 4, where the interval between the first and second impulses in a motoneurone discharge
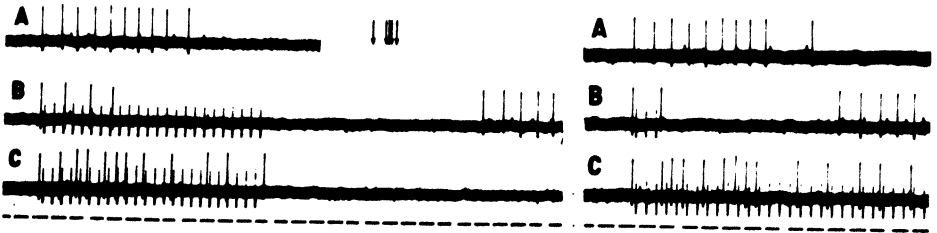
Fig. 1, left. EFFECT ON DISCHARGE OF IMPULSES from a motoneurone of the phrenic nerve, caused by stimulating one of the superior laryngeal nerves at two different intensities. Stimuli (indicated by regularly recurring artifacts) were weaker in *B* than in *C*. In the control record without stimulation (*A*), the arrows indicate the times at which the next inspiratory discharge started in this and in a number of other control observations. *Time is marked at the bottom of this and all other reproductions of original records in $\frac{1}{10}$-second intervals.*

Fig. 2, right. RESPONSE OF A PHRENIC MOTONEURONE to 3 stimuli (*B*) and to many stimuli (*C*), all of the same intensity, applied to one of the superior laryngeal nerves.
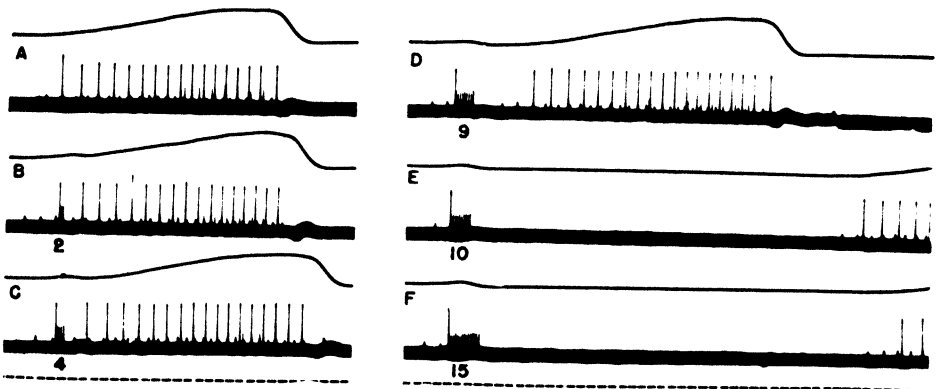
Fig. 3. CHANGES IN THE ACTIVITY OF A MOTONEURONE of the phrenic nerve, caused by the indicated numbers of volleys of afferent impulses over one of the superior laryngeal nerves. Vagus nerves cut. Normal duration of expiration at the time of these observations was 4.0 sec. *The top line in these and many subsequent illustrations is a mechanical tracing of respiratory movements, with inspiration upward.*

is plotted against the number of stimuli. In this graph the interval is expressed as percentage of the normal expiratory pause; this was measured by the average normal time between the last impulse in one inspiration and the first impulse in the next. There was in almost all experiments a certain number of afferent volleys which, when increased by one, resulted in a two- to seven-fold increase in the interval between impulses.

Thus there was usually a well defined transition, as the number of afferent impulses was increased, between a relatively short interruption of the motoneurone discharge and a relatively long interruption. It is interesting to attempt an explanation of this transition in terms of an hypothesis which has been advanced by others concerning the functional organization of the medullary respiratory centers (cf. 4, 7, 8). According to this hypothesis two groups of nerve cells in the medulla oblongata are concerned in respiration. One group, constituting the inspiratory center, discharges impulses during inspiration to the motoneurones supplying the inspiratory muscles. The other group of nerve cells form the expiratory center.
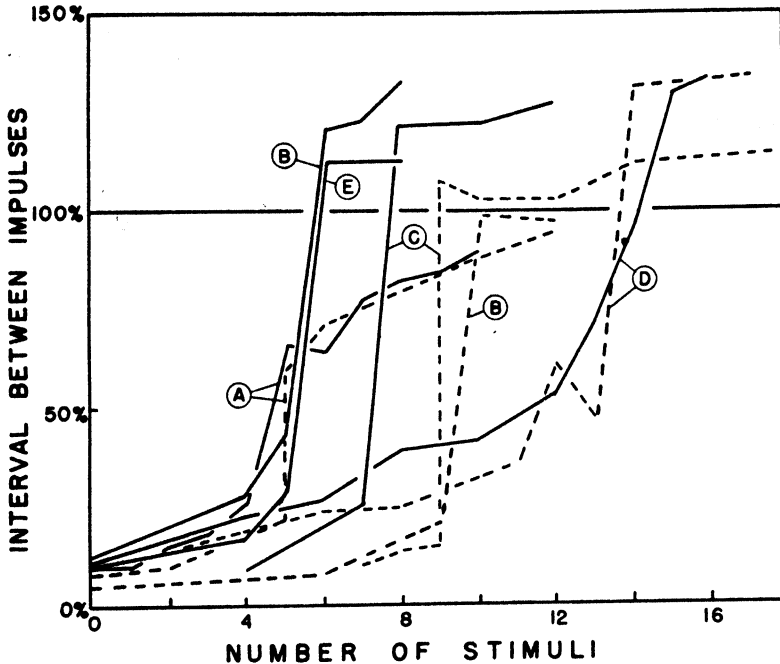
Fig. 4. INTERVAL BETWEEN FIRST AND SECOND IMPULSES in an inspiratory discharge from a phrenic motoneurone, as a function of the number of stimuli applied during the interval to one of the superior laryngeal nerves. Duration of interval is expressed as per cent of the normal duration of expiration. Observations on 5 different animals, indicated by letters. *Solid lines*, experiments with vagus nerves intact; *broken lines*, with these nerves cut. Stimulation at frequencies of 50 to 100/sec. The first impulse was discharged by the motoneurone under observation about 0.4 sec. after start of inspiration for *animal C*, earlier in inspiration for all others.

Each of these two groups is also supposed, when active, to exert an inhibitory effect on the other group, with the result that only one group can be active at a time.

In agreement with this hypothesis, we may suppose that small numbers of afferent impulses over a superior laryngeal nerve temporarily reduce but do not completely stop the activity of the inspiratory center. This reduction lowers the excitatory effect of the center on the phrenic motoneurones and at the same time reduces the inhibitory effect on the expiratory center, but not enough to permit the latter center to become spontaneously active. With larger numbers of afferent impulses, however, we may suppose that the inspiratory center is inhibited to such a degree that the

expiratory center is released and becomes active.   Since this is a situation similar to that supposed to occur normally at the start of expiration, it is not surprising that in many animals the phrenic motoneurones remained quiet for a time comparable to the normal expiratory pause.

These considerations thus show that our observations are not in disagreement with the above hypothesis, which is at present generally thought to account for many of the known facts concerning the respiratory centers.   Our observations might also be explained by other theories; but it does not seem profitable to introduce new lines of speculation until more direct methods have been devised for investigating the respiratory centers.   Therefore, we merely wish to indicate here and elsewhere in this paper that certain findings are not in disagreement with the hypothesis referred to above.

In addition we wish to recognize another proposal which has often been coupled with the foregoing hypothesis, namely, that those afferent impulses which have an inhibitory effect on inspiration do so, not by a direct inhibitory action on the neurones of the inspiratory center, but indirectly through an excitatory effect on the neurones of the expiratory center.   This assumption can also be reconciled with the above observations and with those to be presented later in this paper.   Therefore, we will reserve for discussion at the end of this paper a consideration of whether the afferent impulses establish centrally an inhibitory or an excitatory state.   Until then we will merely assume that the afferent impulses produce some undefined 'state' in the centers, which if sufficiently intense has the effect of stopping insp·'ation and starting expiration.

Afferent impulses later in inspiration.   Effects similar to those described above for stimulation early in inspiration were also observed in some experiments where the afferent volleys were sent in later during inspiration.   That is, a few stimuli delayed and sometimes slightly prolonged the remainder of the motoneurone discharge.   As the number of afferent volleys was increased the delay progressively lengthened, until a critical number was reached at which all subsequent discharge was eliminated. These preparations thus exhibited the same abrupt transition of effect, when the number of afferent volleys was increased, as that described for stimulation early in inspiration (cf. fig. 8, *C* and *D*; also *curves C*, fig. 4).

In other experiments there was also a sharp transition with stimulation late in inspiration, but of a rather different sort (fig. 5).   As before the principal effect observed with small numbers of afferent volleys was a momentary delay in the discharge of the next impulse (fig. 5 *B*).   As the number of stimuli was increased, however, instead of reaching a condition in which *all* discharge subsequent to the stimulation was eliminated, the transition appeared as a dropping of many later impulses, but with 1 to 6 impulses still remaining immediately after the afferent volleys (fig. 5 *C*).   The impulses discharged following stimulation are reflected in the figure by a 'bump' on the descending limb of the mechanical record of respiratory movements, indicating that a significant number of motoneurones took part in the final burst of activity.   This burst of activity could be markedly decreased by applying a single additional stimulus, as shown by both the electrical and mechanical records in figure 5 *D*.

A similar discharge of a few impulses following stimulation was sought in a

number of experiments: no such discharge was ever recorded following stimulation in the first 0.4 seconds of inspiration (6 experiments); impulses appeared in nearly half the observations following stimulation 0.4 to 1 second after the start of inspiration (15 experiments); and the impulses always appeared when the stimulation was delayed more than 1 second (7 experiments). An illustrative example from a single experiment appears in figure 6. Thus the tendency for discharge of a few impulses following stimulation was found to increase progressively during inspiration. No significant alteration was caused in experiments where the vagus nerves were cut, except that tests could then be made later after the start of inspiration, since the latter was prolonged.

In one animal a still different manner of stopping inspiration was observed when afferent impulses were sent into the respiratory centers near the middle of inspiration. Records from this experiment (fig. 7) show that, when increasing numbers of afferent volleys were initiated following the 8th impulse from a certain motoneurone, the number of subsequent impulses was progressively reduced, from a normal of 9 (*record A*), to 6 in *B*, 4 in *C*, 2 in *D* and 1 in *E*. A further increase in number of stimuli (*record F*) did not prevent the discharge of this remaining impulse, although elimination of the 'bump' on the mechanical record (compare *records E* and *F*) indicated that the additional volley of afferent impulses had a marked effect on the activity of other motoneurones. The progressive shortening of inspiration observed in this animal stood in marked contrast to the usual effects, in which inspiration shortened suddenly as the number of afferent volleys was increased.

Threshold for stopping inspiration. In accordance with the observations described above, it was possible to define a threshold for stopping inspiration in terms of the minimum number of stimuli of given strength and frequency required to stop the discharge from phrenic motoneurones. When the discharge could be stopped with no impulses appearing after the stimulation (as in figs. 3 and 8), then the threshold was defined as the number of stimuli corresponding to the mid-point of the steepest portion of curves relating interval to number of stimuli (such as the curves in fig. 4). When a few impulses followed the minimum number of stimuli which stopped inspiration (as in figs. 5–7), then the mechanical records were examined to see whether the impulses discharged after stimulation were sufficient to cause a 'bump' on the descending limb, such as that appearing in figures 5 C and 7 E. When such an inflection occurred it always vanished when the number of stimuli was increased by one or two (figs. 5 D and 7 F), even though the particular motoneurone under observation sometimes still fired briefly after the stimulation. Under these circumstances the threshold for stopping inspiration was assumed to lie midway between the minimum number of impulses which prevented the 'bump' on the mechanical record and the maximum number which failed to do so.

With the threshold for stopping inspiration thus measured by the number of afferent volleys, it was possible to determine quantitatively how the threshold varied under altered conditions, provided the intensity of stimulation and hence the sizes of the afferent volleys were kept constant.

Changes in threshold during inspiration. More afferent volleys were required to stop inspiration at its beginning than when the afferent volleys were initiated later in

the cycle. This is illustrated in figure 8. In this experiment, 8 afferent volleys, immediately after the first impulse from a certain phrenic motoneurone, caused a
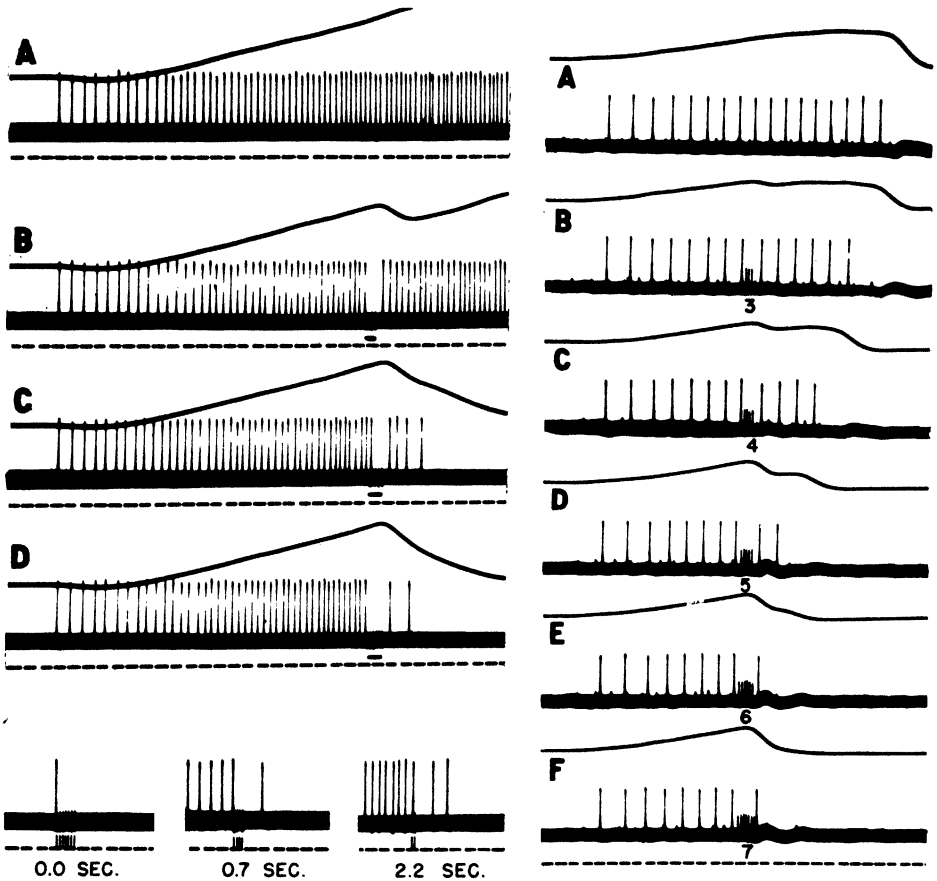
Fig. 5, upper left. EXAMPLE OF AN EFFECT ON PHRENIC MOTONEURONE DISCHARGE caused by stimulation late in inspiration. Following stimulation in C and D a few more impulses were discharged before activity ceased. Number of afferent volleys over one of the superior laryngeal nerves: 4 in B, 5 in C and 6 in D. Time of stimulation indicated by short horizontal line above timer. Vagus nerves cut. (Records taken while animal was breathing 5% CO_2, 95% O_2.)

Fig. 6, lower left. MANNER OF STOPPING PHRENIC MOTONEURONE DISCHARGE in a typical preparation. Minimum numbers of stimuli required to curtail activity were applied to one of the superior laryngeal nerves at indicated times after start of inspiration. The first (and only) impulse discharged appears in left record, but merely the end of activity is shown in the others. At the time these records were taken inspiration normally lasted more than 3 sec. Short vertical lines above time record indicate stimuli. Vagus nerves cut.

Fig. 7, right. UNUSUAL EFFECT OF AFFERENT IMPULSES over a superior laryngeal nerve on activity of a phrenic motoneurone. Discharge from the motoneurone was progressively shortened as number of afferent volleys was increased. Numerals indicate numbers of stimuli, which were applied at times revealed by artifacts. Vagus nerves cut.

moderate delay of the second impulse. Increasing the number of stimuli to 9 sufficed to stop activity for a time approximating normal expiration. When the afferent

volleys were sent in, not at the start of inspiration but about ½ second later, only 7 volleys rather than 9 were required to stop inspiration.

The change in threshold for stopping inspiration was demonstrated in another way in the experiment shown in figure 9. Here 5 stimuli, at a certain intensity and frequency, failed to stop the discharge from a phrenic motoneurone when applied early in inspiration (*records B* and *C*). When applied slightly later, similar stimuli did curtail the activity (*records D* and *E*). A similar decrease in threshold for stopping inspiration was observed by Boyd and Maaske (2) as stimuli to the vagus nerve were moved later in inspiration.

In several experiments the threshold for stopping inspiration was systematically determined at a number of times after the start of inspiration, with the results summarized graphically in figure 10 *A*. The progressive decline in threshold was clearly demonstrated in all experiments. Moreover, there was a surprising simplicity about
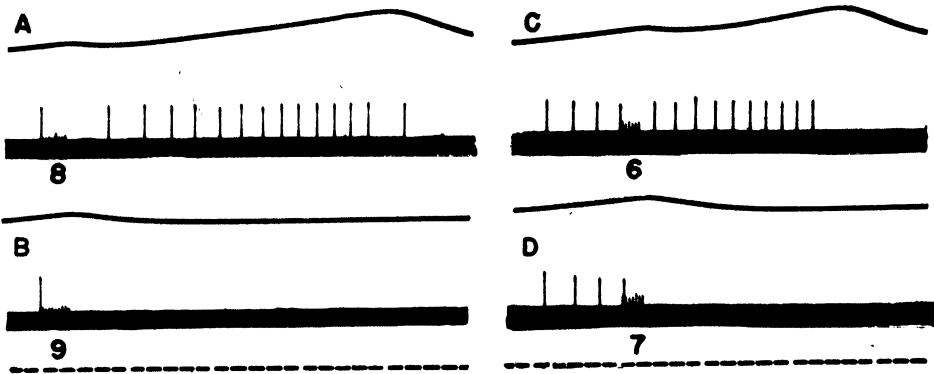
Fig. 8. NUMBER OF AFFERENT VOLLEYS over one of the superior laryngeal nerves required to stop discharge from a phrenic motoneurone was greater at start of inspiration (records on left) than later in the cycle (records on right). Numerals indicate number of stimuli, which were all of same strength. Vagus nerves cut.

this decline: in all experiments, within the accuracy of measurement, the threshold for stopping inspiration fell *linearly* during inspiration.

The several experiments have been further correlated in figure 10 *B*. In order to reduce all observations to a comparable time scale, the time from the start of inspiration to the last stimulus was expressed as percentage of the average duration of normal inspiration in each series of observations. The threshold number of impulses was also expressed in percentage by adjusting the scale of ordinates for each experiment so that the straight lines drawn in figure 10 *A* all passed through a threshold value of 100 per cent at the start of inspiration. It can be seen that, after these adjustments of coordinates, all the points fell near the single straight line drawn in figure 10 *B*. The fairly symmetrical scatter of points on the two sides of the line suggests that the deviations may be due to experimental errors of measurement and that the linear relationship may actually be followed very precisely.

The progressive decline in number of afferent impulses required to stop inspiration is evidence that changes in the respiratory centers, which eventually terminate inspiration, develop gradually throughout this phase of respiration. Further signifi-
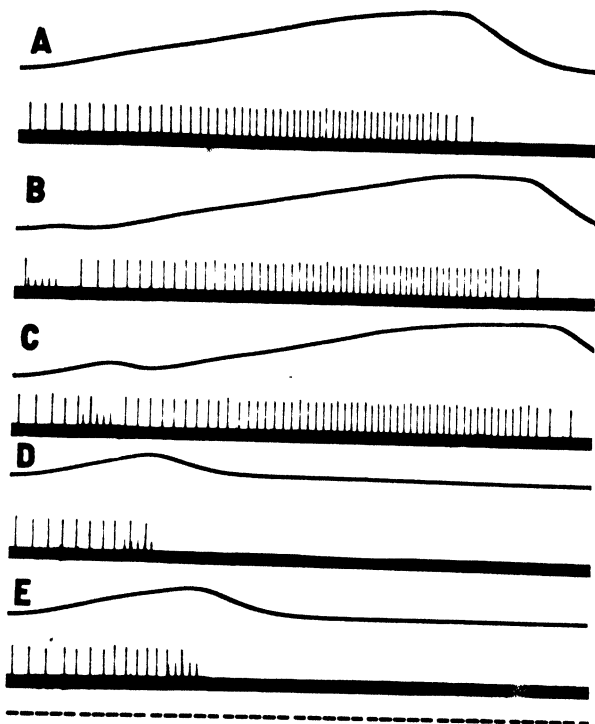
Fig. 9. WHEN 5 STIMULI OF SUITABLE STRENGTH were applied to one of the superior laryngea nerves early in inspiration (*B* and *C*), they failed to stop the discharge from phrenic motoneurones. Later in the cycle similar afferent volleys cut inspiration short (*D* and *E*). Vagus nerves severed
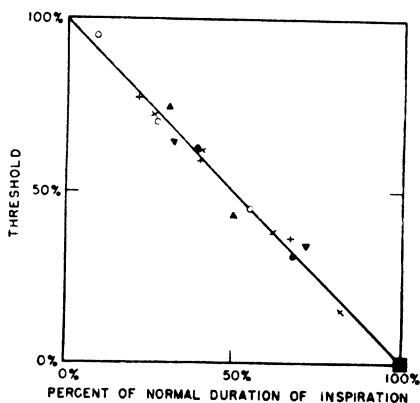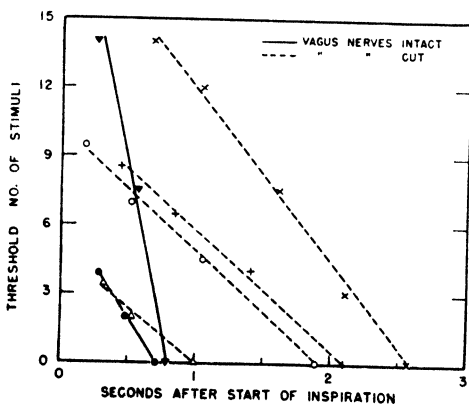
Fig. 10. THRESHOLD FOR STOPPING INSPIRATORY ACTIVITY by stimulating one of the superior laryngeal nerves at various phases of inspiration. Six series of observations on 5 different animals. Frequency of stimulation about 50/sec. in each. In *A* the number of afferent volleys required to stop inspiration is plotted against time from the start of inspiration to final stimulus. All lines drawn in this plot are straight. In *B* all straight lines of *A* have been made to coincide by suitable adjustments of scales of coordinates. See text for further explanation.

cance and the possible nature of these changes will be considered in the final section of this paper.

Quite puzzling is the constant rate at which the threshold declines, as indicated by the linear relationship shown in figure 10.   Such a constant rate of change throughout the entire measurable course of a biological process is unusual.   Possibly it is a result of the combination of several non-linear factors which add together to determine the measured change.   It is, however, surprising that the several factors should by coincidence combine so as to give a linear result in each of several different preparations.   Therefore, we suggest that this linearity may have a simple explanation and, when understood, will reveal an important detail concerning the mechanisms of the respiratory centers.

Effects of Afferent Impulses During Expiration

The foregoing experiments offer direct evidence of progressive changes occurring in the respiratory centers during inspiration.   Evidence of comparable progressive
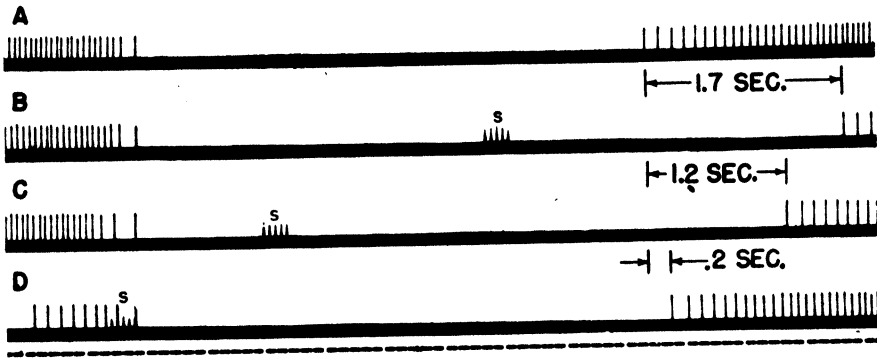
Fig. 11. LENGTHENING OF EXPIRATORY PAUSE, caused by a few volleys of afferent impulses over a superior laryngeal nerve.   *A* is a control record without stimulation, showing end of one phrenic motoneurone discharge and start of the next.   This record was carefully selected to show an expiratory pause of average duration.   Five stimuli at fixed intensity were applied during expiration in *B* and *C*, during inspiration in *D*.   Timing of stimuli indicated by stimulus artifacts appearing in each record at *S*.   Vagus nerves cut.

changes occurring during expiration was obtained from interpretation of the effects next to be described.   These effects were observed when afferent impulses were sent into the respiratory centers during expiration.

A few volleys of afferent impulses over a superior laryngeal nerve, during expiration, delayed the start of the next inspiration.   This is illustrated in figure 11. A normal expiratory pause in the absence of stimulation is shown in *record A*.   In *record B*, 5 stimuli were applied to the superior laryngeal nerve during the latter part of expiration, with the result that the next inspiration was delayed for 1.7 seconds. In *record C* of figure 11, the stimuli were applied earlier than in *B*, and the delay of inspiration was less.   Even when the stimuli were further advanced so that they fell early in the preceding inspiration (*record D*), there was still a detectable prolongation of the expiratory pause.   The progressive decrease in this prolongation of expiration, caused by moving the stimuli earlier in the respiratory cycle, is shown more completely by the data from two experiments plotted in figure 12.   Here, as in so many

other observations in this paper, no obvious qualitative difference was caused by cutting the vagus nerves.

In interpreting the graphs of figure 12 one may assume, as we have done before, that the afferent impulses produce a certain altered 'state' within the respiratory center, which has the effect of inhibiting inspiratory activity. This state is progressively dissipated with the passage of time. Accordingly, the delay of the next inspiration is a quantitative measure of the residue of this state remaining at the time when inspiration would normally start. Moreover, the change in inspiratory delay with a given change in timing of the stimuli is an indication of the rate of decline of
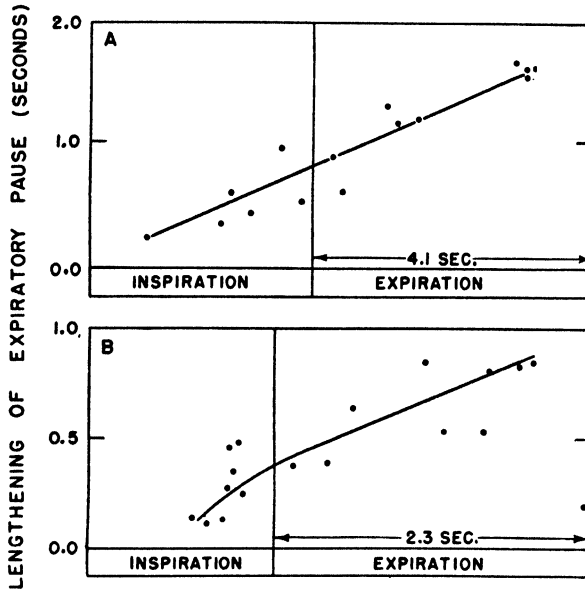
Fig. 12. INCREASE IN DURATION OF EXPIRATION (ordinates) caused by afferent volleys over a superior laryngeal nerve at various times during the respiratory cycle. Abscissae indicate times at which stimuli were applied. Lengthening of expiration was calculated by comparison with average of many normal expirations. Data from 2 different animals. Number, frequency and strength of stimuli were kept constant throughout the observations in each. Vagus nerves cut in *A*, intact in *B*. The numbers of stimuli were sufficient to stop inspiration when applied at the time of each point plotted during inspiration, but did not stop inspiration when initiated earlier.

the state. It is with this rate of decline that the remainder of this section is concerned.

In order to measure the rate of decline of the state produced by afferent impulses the delay of inspiration caused by afferent volleys at the end of expiration was compared with the smaller delay caused by similar volleys one second earlier. For this purpose the delays caused by stimulation at these two times were read from graphs such as those in figure 12, the effect of stimulation exactly at the end of expiration being determined by extrapolation. The percentage differences between the two delays varied considerably from animal to animal. It was discovered, however, that the differences were closely correlated with differences between the normal durations

of expiration in the several animals (fig. 13, *curve A*). The longer was the normal expiration, the less was the change in effect due to the one second change in timing of the afferent volleys.

The simplest interpretation of this observation is that the state, established centrally by the afferent impulses, declines most slowly in those animals with longest expirations. The significance of this finding will be discussed presently.

An even simpler relationship between the rate of decline of the central state and the length of expiration was suggested by comparing the delays of inspiration caused by equal numbers of afferent volleys sent in at the start and at the end of expiration.
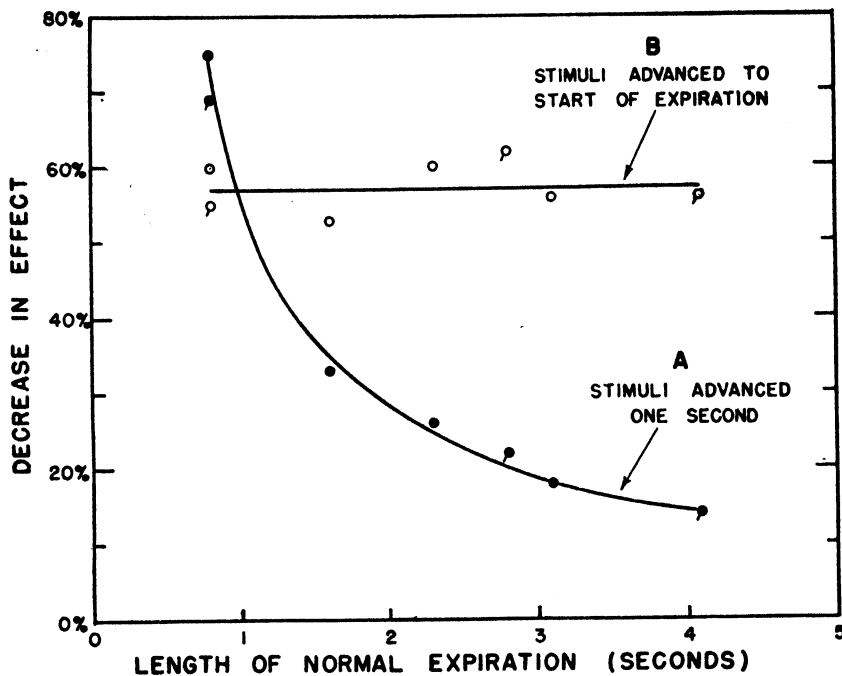
Fig. 13. DECREASE IN DELAY OF INSPIRATION when stimuli to one of the superior laryngeal nerves were moved from the end of expiration to the indicated earlier times. Tails drawn on some of the points identify experiments in which vagus nerves were cut.

In a manner similar to the preceding analysis, the percentage reduction in effect was calculated when the stimuli were advanced from the end to the start of expiration. This gave the very surprising result that the reduction in effect was about the same in all animals (fig. 13 *curve B*). In other words, if a certain number of afferent impulses delayed inspiration by a given amount when sent in at the end of expiration, then the same number of afferent impulses sent in at the start of expiration always produced a delay which was about 55 to 60 per cent shorter. It must be emphasized that in advancing the stimuli from the end to the start of expiration, the timing of the afferent volleys was in two cases changed by less than one second and in another by over four seconds, yet the percentage reduction of effect was about the same in

these, as in other animals with intermediate lengths of expiration. The simplest interpretation of this last observation is that the central state, produced by the afferent impulses, declined by about the same percentage in all animals during a time equal to the normal expiratory pause.

A possible explanation of the relationships just described can be given in terms of an hypothesis concerning the natural control of respiratory rhythmicity. Let it be assumed *a*) that in natural breathing a certain central state is developed progressively during inspiration, *b*) that inspiration stops when this state reaches a certain level and *c*) that inspiration starts again when this state has declined to a lower limiting level. Accordingly the length of the expiratory pause is determined by the time required for this state to fall from one critical level to another. Therefore, the longest expirations may be expected in those animals with slowest decline of the proposed naturally-occurring central state.

For simplicity let it further be assumed *d*) that the naturally-occurring central state is identical in nature with the state produced by afferent impulses. Accordingly the state produced experimentally by afferent nerve stimulation, like that which occurs naturally, should also decline most slowly in those animals with longest inspirations. This is in agreement with the findings expressed by *curve A* of figure 13.

To account for the uniform decrease in effect when the afferent impulses are moved from the end to the start of expiration (fig. 13, *curve B*), it is necessary to assume further *e*) that in natural breathing the two critical levels of the proposed state, at which inspiratory activity stops and starts, are about the same in all animals, or are at least in approximately a constant ratio to one another. Accordingly the percentage decline in the state during a time equal to one expiration would be the same for all animals. This is in agreement with the findings presented by *curve B* of figure 13.

Nature of the Central State

Our observations have been found to agree with the concept that respiratory rhythmicity is brought about by the progressive development during inspiration of a central state, which stops inspiration when a critical level is reached and which subsides gradually during expiration. The progressive rise during inspiration is in agreement with the decrease in threshold for stopping inspiration by afferent impulses (fig. 10). The gradual subsidence during expiration is in agreement with the differences in effects produced by afferent impulses at different times during expiration and, particularly, with the relationship between these differences and the duration of normal expiration (fig. 13).

This concept of a waxing and waning central state is not new. It has been employed to explain how impulses, from pulmonary receptors and from the pneumotaxic center, influence the respiratory rate by exerting an inhibitory effect which increases progressively during inspiration. Certainly, the proposed state can be developed by factors other than afferent impulses from the lungs, for we have found that no qualitative change is caused by sectioning the vagus nerves.

Our observations not only furnish strong experimental support for this hypothesis that respiratory rhythmicity results from a fluctuating central state, but also

suggest a certain addition to it, namely, that the state declines slowly, even after the agent which develops it has ceased to act.   This was shown by the long persistence of the inhibitory effect of a few volleys of afferent impulses during expiration.   There is also evidence that the central state persists, at least for a short time, when it is
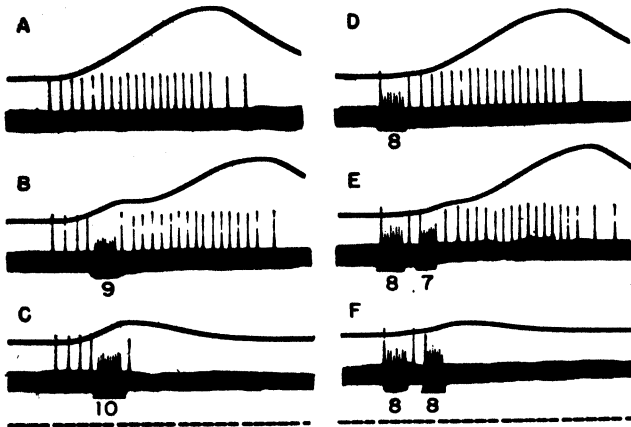
Fig. 14. REDUCTION OF THRESHOLD for stopping inspiration, caused by a few conditioning volleys of afferent impulses.   Numerals indicate numbers of stimuli.   Without conditioning, the threshold was between 9 volleys (*B*) and 10 (*C*).   After 8 conditioning volleys (appearing alone in *D*), the threshold was between 7 (*E*) and 8 (*F*).
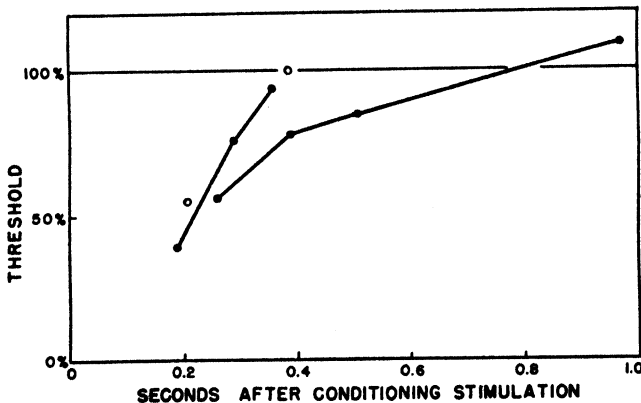
Fig. 15. REDUCTION OF THRESHOLD for stopping inspiration, following conditioning volleys of afferent impulses sent in at the start of inspiration.   Experiments similar to that of fig. 14, the conditioning and testing stimuli being applied to one superior laryngeal nerve at a frequency of 50/sec.   *Abscissae:* time from end of conditioning stimuli to end of testing stimuli; *ordinates:* threshold as percentage of control threshold found at the same time after the start of inspiration in the absence of conditioning stimulation.   *Filled circles*, data from 2 animals with vagus nerves cut; *open circles*, another animal with vagus nerves intact.

produced by impulses sent in during inspiration.   This has been revealed by the after-effects of impulses too few in number to stop inspiration.   It was found that such volleys of impulses were followed by a reduction in threshold for stopping inspiration which lasted for at least several tenths of a second (figs. 14 and 15).   A

similar enduring effect on threshold was found in one experiment when the conditioning impulses entered over one superior laryngeal nerve and the testing impulses over the other.

The nature of the proposed central state within the respiratory centers has not been determined. One possibility which must be considered is an inhibitory state at the inspiratory neurones in the medulla oblongata, which waxes during inspiration and wanes during expiration. This suggestion, however, encounters certain difficul-
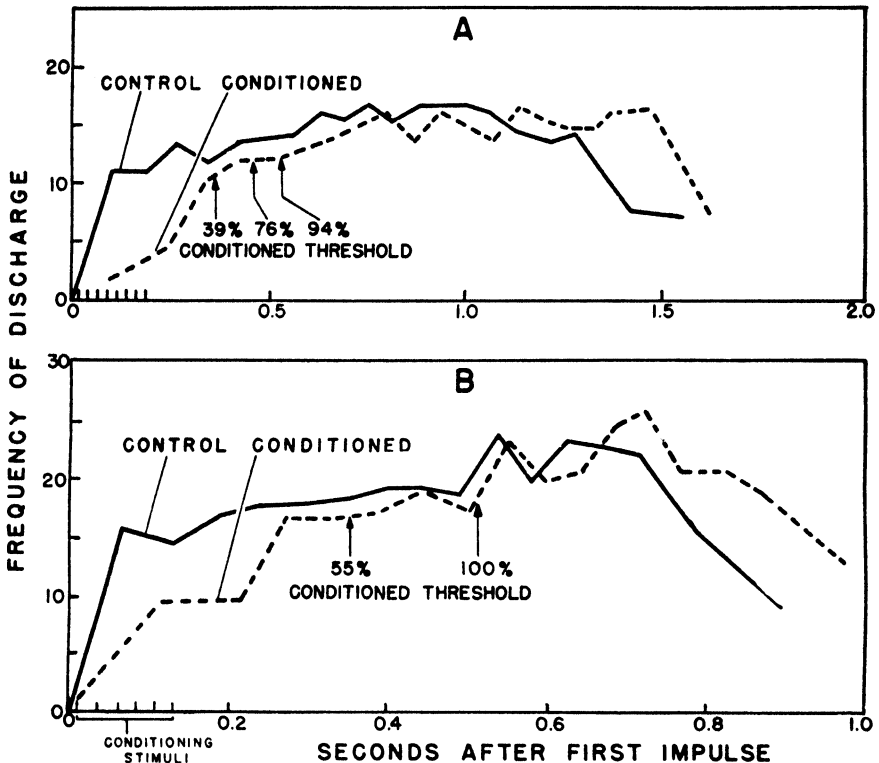
Fig. 16. FREQUENCY OF DISCHARGE from a phrenic motoneurone in a normal inspiration and during an inspiration modified by a few conditioning stimuli applied to a superior laryngeal nerve immediately after the first impulse in inspiration. Ordinates of curves are reciprocals of successive intervals between motoneurone impulses. *A* and *B*, for two preparations represented in fig. 14; *A* with the vagus nerves cut, *B* with these nerves intact. Thresholds for stopping inspiration following conditioning stimulation are indicated in percentage of normal threshold, at several times after the start of inspiration.

ties. In the first place one would expect that during natural breathing an incrementing inhibitory state would be reflected in a progressive slowing of the frequency of discharge from motoneurones supplying muscles of inspiration. On the contrary, it is known that during each inspiration there is a progressive increase in both the frequency of discharge from the individual motoneurones and in the number of motoneurones in action (3, 4). Therefore, it is suggested that the relevant fluctuating state be sought elsewhere than at the central inspiratory cells.

In agreement with this conclusion is evidence that the central state produced by stimulating an afferent nerve, which seems so closely related to the naturally fluctuating state, is also not associated with the inspiratory neurones in the medulla. Experiments already presented indicate that during inspiration the state produced by afferent impulses persists for several tenths of a second (figs. 14 and 15). Yet there was no corresponding persistent change in the frequency of discharge from a phrenic motoneurone. This is shown by the frequency analyses reproduced in figure 16. It is true that the frequency was slightly lower at the time of the tests after conditioning than it was at the same time without conditioning. But the frequency curves are approximately parallel at the time of the threshold tests: they do not approach each other as the threshold for stopping inspiration approaches normal. This lack of persisting effect on motoneurone discharge suggests a similar lack of persisting effect on the activity of cells of the inspiratory center, for Pitts (6), by stimulating the center while recording from a motoneurone, has revealed a close relationship between the activity of these two sets of cells. Consequently, it is unlikely that the persisting state produced by afferent impulses involves the inspiratory cells of the respiratory center.

Another possibility is that the naturally fluctuating central state, and the central state produced experimentally by afferent impulses, occur as excitatory changes at the expiratory cells of the medulla. Such an excitatory state could indirectly exert an inhibitory influence on the inspiratory cells, since the work of other investigators has already suggested the existence of appropriate neuronal connections within the medulla oblongata. This hypothesis concerning the central state does not encounter the difficulties found by assuming that the state affects the inspiratory cells directly, since a subthreshold excitatory state could develop at the expiratory neurones without influencing the activity of inspiratory cells. The subthreshold state could, however, facilitate the excitation of expiratory neurones by afferent impulses and thus account for the observed reductions in the threshold for stopping inspiration.

Finally it should be recognized that there are undoubtedly other possible locations for the fluctuating central state than those which have been discussed. Our experiments indicate that the state is not at the inspiratory neurones in the medulla. Obviously, further investigation is required to define as well as to locate this central state, which we have been forced to assume in order to account for the observations presented in this paper.

SUMMARY

1. Progressive changes in the respiratory centers, occurring during inspiration and during expiration, were revealed by quantitative variations in the effects of afferent impulses sent into the centers at various times during the respiratory cycle. The afferent impulses were initiated by electrical stimulation of the superior laryngeal nerves of anesthetized cats and the effects observed by recording the discharge of impulses from single phrenic motoneurones.

2. One or two volleys of afferent impulses, initiated immediately after the first

impulse discharged by a motoneurone at the start of inspiration, slightly delayed the discharge of the second impulse. As the number of afferent volleys was increased, the delay of the second impulse became progressively longer until a critical number of volleys was reached, at which the delay of the second impulse abruptly increased many-fold. This larger delay was usually similar in duration to the normal expiratory pause. When the afferent impulses were initiated later in inspiration, a sufficient number of volleys sometimes abruptly stopped the motoneurone activity, sometimes a few motor impulses were discharged following the afferent nerve stimulation before inspiration was brought to a premature close. The threshold number of afferent volleys required to stop inspiration declined progressively throughout the inspiratory phase of respiration. Threshold was found to be a linear function of time in each of the six preparations studied.

3. A few volleys of afferent impulses during expiration delayed the start of the next inspiration. Following a given number, frequency and intensity of stimuli, the delay of inspiration was less the earlier in expiration the afferent impulses were initiated. The delay was reduced more by a one-second advance in timing of the stimuli in animals with short expiratory pauses than in animals with longer pauses. On the other hand the delay of inspiration was about equally reduced by advancing the stimuli from the end to the start of expiration, in all preparations, regardless of the duration of expiration.

4. These findings can be explained by assuming that the respiratory center is influenced by an as-yet-undefined state somewhere within it which is developed progressively during natural inspiration and subsides gradually during expiration. Activity of inspiratory neurones in the center is assumed to stop when this state attains a certain level and to resume when another, lower, level is reached. The effects of afferent impulses initiated by electrical stimulation may then be explained by assuming that these impulses cause additions to the naturally-occurring central state. Thus the progressive decline in threshold for stopping inspiration may be due to the gradual development of the proposed state during inspiration, so that a smaller contribution is required from the experimentally-initiated impulses in order to terminate inspiration.

The delay in start of inspiration, caused by afferent impulses during expiration, is explained by assuming a persistence of the central state developed by these impulses. Other evidence of such persistence was obtained by the finding of summation between the effects of two appropriately-spaced groups of afferent volleys. The relationships between the natural length of the expiratory pause and the rate of decline of the central state can also be explained.

5. It is possible that contributions to the naturally-occurring central state may normally be caused by afferent impulses from the lungs by way of the vagus nerves, but the state can also be developed by other factors, since no qualitative differences were found after sectioning the vagus nerves. During persistence of the central state developed by afferent impulses there was no corresponding reduction in frequency of discharge from phrenic motoneurones. This suggests that the central state is not an inhibitory state associated with the inspiratory cells in the respiratory centers. A possible explanation of the observed phenomena may, however, be

made by assuming that an excitatory state is developed at the central expiratory neurones.

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SUDDEN VASOCONSTRICTION IN DENERVATED OR SYMPATHECTOMIZED PAWS EXPOSED TO COLD

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WHEN an animal is placed in a cold room the skin of the extremities becomes cool owing to reflex vasoconstriction of the arteriovenous anastomoses (1). A sympathectomized or denervated extremity tends to remain warm because the vasoconstrictor pathways have been interrupted. This initially maximal vasodilatation does not, however, persist indefinitely. Starting a few hours after their nerves are severed, the blood vessels begin gradually to regain their 'tone' (2) and after a few days may constrict in cool surroundings (3). Such vasoconstriction after total denervation or sympathectomy has been described in man (4, 5), in monkeys (6) and in rabbits (3), but there is incomplete agreement with respect to the responsible mechanism (3–7).

It was noted by us (8) during the course of other studies in which a dog was placed in a room at 10°C., that the skin temperature of a sympathectomized paw, after remaining elevated for somewhat over an hour, suddenly fell sharply, and continued to decline until reaching essentially the same temperature as the opposite, normally innervated paw in which vasoconstriction was known to be nearly maximal. To our knowledge, an abrupt vasoconstriction occurring in sympathectomized or denervated extremities exposed to cold has not been reported previously, though a somewhat similar effect has recently been observed by Ungley (9) in cases of 'trench foot' of man, a condition in which degeneration of nerves occurs.

The present paper reports studies on the effects of prolonged cold on skin temperatures of sympathectomized or denervated vessels in the paws of cats and dogs. Sudden vasoconstriction appeared when the extremity was cooled below a certain 'critical temperature' and was complete enough to reduce blood flow to approximately one-tenth its former value. Reasons are given for attributing this reaction to the development of sensitivity to cold on the part of vascular smooth muscle after sympathectomy and total denervation.

METHODS

Observations were made *a*) repeatedly on one unanesthetized, trained dog (Series I), *b*) on 7 lightly anesthetized cats while the whole body and the paws were exposed to cold (Series II) and *c*) on 9 lightly anesthetized cats while the body was kept warm and the paws only were exposed locally to cold (Series III).

In those animals, as indicated in the tables, in which sympathectomy was performed, the lumbar sympathetic chain with its ganglia was removed from the level of the renal artery to the bifurca-

tion of the aorta, thereby providing an essentially preganglionic sympathectomy of the lower hind limb.   In another group of animals the sciatic nerve was sectioned close to its point of emergence from the sciatic notch and proximal to all branches of the nerve.   In a third group of animals, more extensive procedures aimed at total denervation of the leg were carried out as described below.

At periods ranging from 0 to 130 days after operation the effect on skin temperature of prolonged exposure to a cold environment was tested.   The trained anesthetized dog was supported by slings in a wheeled Pavlov stand.   Anesthetized cats, given 22.0 to 32.5 mg. of pentobarbital (Nembutal, veterinary) intraperitoneally, a dose which did not abolish shivering or reflex vasoconstriction, rested with their ventral surface on a wooden stand so that the paws hung freely in the air.

Skin temperatures were measured on the dorsal surfaces of the paws at the base of the digits by iron-constantan thermal junctions applied under loose adhesive tape or by collodion and a wisp of cotton.   The reference junctions were immersed in oil in a thermos flask, or in boiling ether with a reflux condenser, each thermocouple circuit being led independently to a galvanometer through a selector switch so that readings could be made rapidly in rotation, free of complicating skin potentials.   Repeated calibrations indicated that the measurements were accurate to within ±0.5 degrees C.   Rectal temperature was determined by a mercury thermometer left continuously in place or by means of a thermocouple.

Each experiment was started with maximal dilatation of the cutaneous vessels of the paws by placing the animal in a thermoregulated room at 30°C. ±0.5°C.   In some instances one or two electrical heating pads were placed around the body to hasten the abolition of vasoconstrictor tone. When vasodilatation was complete, as indicated by skin temperatures consistently between 34 and 36.5°C., the animals were taken into another thermoregulated room set at the desired temperature between 25° and 0°C ±1.0°C.   Skin temperatures were measured at intervals of five minutes or less.

For localized cooling of the paws alone and to provide more rapid changes in the temperature of the air surrounding the paws, a 30 x 20 x 12 inch box was lined with copper pipes connected to a source of brine at −13°C.   A fan circulated air at low velocity through the narrow space between an inner sheet metal lining, on three sides of the box, and the brine pipes on the walls of the box. The desired constant temperature was obtained by means of a thermostatically controlled electric heater used in conjunction with manual regulation of the flow of brine.   The cat was supported above the box by a sling, its paws projecting into the box through holes in a felt diaphragm in the cover. The box was located in one of the thermoregulated rooms so that the animal's body could be surrounded by air at any desired temperature, whereas the paws, or the entire legs if desired, could be surrounded by air at any other temperature.   In most cases, the room was kept at 30°C. and the box, initially at 30°C., was gradually cooled by steps to temperatures sometimes as low as −5°C. Skin, rectal and box temperatures were measured thermoelectrically.

Changes of skin temperature were translated into approximate changes of blood flow by means of Burton's 'Thermal Conductivity Index,' ('TCI') = $(T \text{ skin} - T \text{ air})/(T \text{ rectum} - T \text{ skin})$ (in which ratio 'T' stands for temperature).   The declining portions of the skin temperature curves were also plotted semi-logarithmically to determine the abruptness and degree of constriction by a) the straightness of the line and b) the thermal time constant 'k' as will be described more fully below.

I. General Characteristics of the Sudden Vasoconstriction

Figure 1, (*upper*) shows in the unanesthetized dog the usually described response observed during cooling after lumbar sympathectomy.   In the warm room kept at 30°C., skin temperatures were 34°C. or more.   When the dog was exposed to air at 15°C., the skin temperature of the normal extremities approached environmental temperature as vasoconstriction developed, but the temperature of the sympathectomized extremity remained relatively constant at 26 to 27°C.   When the dog was exposed to a temperature of 10°C., (fig. 1, *lower*) the sympathectomized extremity remained relatively warm at first, but when its temperature reached 24°C., it began, as a result of sudden vasoconstriction, to cool abruptly, producing a distinct 'angle'

in the plotted temperature curve. Thereafter, the temperature of the sympathectomized paw declined along a curve similar in shape and time relations to the skin temperature curve of the vasoconstricted, normally innervated opposite paw.

In these and other similar experiments, throughout a considerable period prior to the sudden vasoconstriction, there was often a gradual decline in the skin temperature of the sympathectomized paw, indicating gradual vasoconstriction. In addition, fluctuations in the skin temperature of one to two degrees often appeared just before
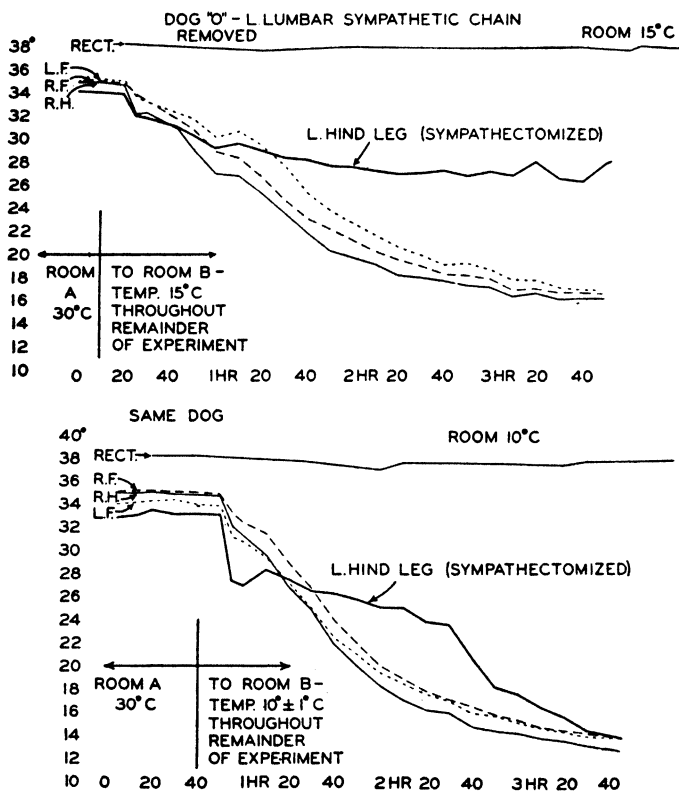
Fig. 1. TEMPERATURE CHARTS, in °C., from two experiments performed on different days on an unanesthetized dog in a cold room. Skin temperatures in these and subsequent experiments were determined on dorsal aspect of paws. *Upper chart.* With room at 15 C. vasodilatation persisted. *Lower chart.* With room at 10°C. sudden vasoconstriction, indicated by a sudden fall in skin temperature, occurred in the sympathectomized paw.

or after the sudden vasoconstriction. However, once the temperature of the sympathectomized paw had abruptly declined more than two or three degrees, in most experiments it did not rise again to its previous level, but continued to fall.

Following the sudden vasoconstriction and after the temperature of the paw had leveled off at just above environmental temperature, sudden vasodilatation, indicated by a sudden rise in skin temperature, was observed in three experiments (two on *animal 30*, table 3) during gradual warming of the air surrounding the paw (fig. 4).

II. *Evidence for a 'Critical Temperature' at Which Sudden Vasoconstriction Occurred*

The three experiments charted in figures 1 and 2 provide data indicating that the sudden vasoconstriction did not occur until the skin of the sympathectomized paw was cooled to a certain 'critical' temperature. Thus, with the room at 15°C., (fig. 1, *upper*) the gradient of temperature between the body (rectal temperature) and the room was such as to cool the vasodilated paw to somewhat above 26°C., but the temperature of the paw never fell below that value and the skin of the paw remained vasodilated. However, with the room at 10°C., (fig. 1, *lower*) the correspondingly greater cooling gradient sufficed to lower the skin temperature of the vasodilated paw to approximately 24°C., at which temperature sudden vasoconstriction occurred.

Further evidence for the existence of a critical temperature for vasoconstriction is provided by the experiment charted in figure 2, performed after both lumbar sympathetic chains had been removed from the dog. Sudden vasoconstriction occurred in the right hind paw after three hours in the cold room, but vasodilatation
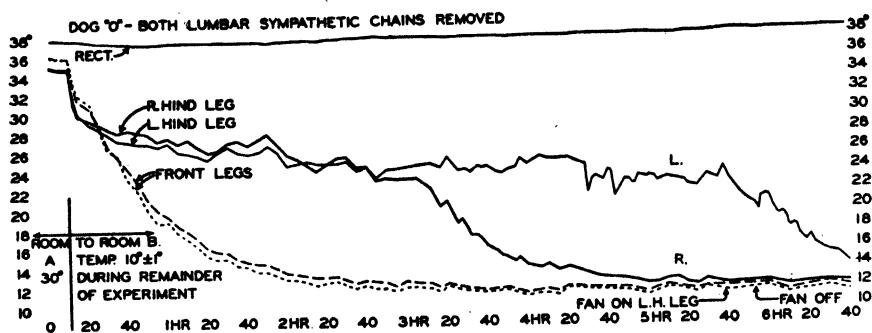
Fig. 2. FOLLOWING REMOVAL OF BOTH LUMBAR SYMPATHETIC CHAINS, sudden vasoconstriction occurred in right hind paw after three hours in the cold room, but did not occur in left hind paw until it was cooled for a brief period by an electric fan.

was still present in the left hind paw, apparently less 'sensitive' to cold, even after five hours in the cold room. At this point, the left hind paw and leg were cooled for 15 minutes by means of an electric fan, following which sudden vasoconstriction occurred.

That it was the skin temperature of the operated paw, rather than the room or rectal temperature, which determined whether or not sudden vasoconstriction occurred is indicated by reference to the columns entitled 'Temperatures at Time of Sudden Vasoconstriction', in tables 1, 2 and 3. These temperatures were taken in each experiment at the time of the 'angle' in the skin temperature graph. It will be noted that the absolute values of room, box or rectal temperatures bear no consistent relation to the sudden vasoconstriction except insofar as they together constitute a gradient of temperature capable of cooling the operated paw. In the experiments included in the tables, this gradient was adjusted so as to cool the paw gradually to its critical temperature. Such experiments were characterized by the usual vasodilatation of sympathectomy or denervation, which persisted, as indicated by an elevated though very gradually declining portion of the skin temperature graph,

until the sudden vasoconstriction occurred. In those experiments, not included in the tables, performed at low temperatures, e.g. 0° to 5°C., the graph of skin temperature for the operated paw closely resembled the graphs for the normal paws. Thus, vasoconstriction occurred promptly in the operated as well as in the normal paws, apparently because the operated paw was cooled below its critical temperature very soon after the animal entered the cold room. In most experiments the time of onset of shivering was noted and the intensity was graded on an arbitrary scale of 0 to 5. There was found to be no correlation between the time of onset or severity of shivering and the time of sudden vasoconstriction.

TABLE I.   SERIES I.   UNANESTHETIZED DOG—COLD ROOM

ANIMAL NO.	OPERATIVE PROCEDURE	DAYS POST-OP.	TEMP. AT TIME OF SUDDEN VASOCONS., °C.			THERMAL CONDUCTIVITY INDEX		STRAIGHTNESS OF SEMI-LOG PLOT	TIME CONSTANT k, MIN.	
			Room	Rectal	Skin, paw	Before vasocons.	After vasocons.		Normal paw	Op. paw
Dog 0	Removal 1. lumbar symp. chain	12	10°	39.6°	23.8°	0.89	0.12	++++	50	48
"	Removal rt. lumbar symp. chain 9 mos. later	10	10°	37.9°	L. 20–26° R. 21–23°	1.4 0.9	0.12 0.12	++ ++	46	33 36
"	Same	12	15°	37.8°	L. R. ?(fan used)	1.7 1.65	0.10	++++	48	45
"	Same	24	10°	38.0°	L. ?(fan used) R. 22.4–23.2°	0.80 0.87	0.08	++ ++++	41	42 32
"	Same	130	5–3°	37.4°	L. 20° R. 20–22°	1.10 1.40	0.02	+++	44	
Averages.................................					22.1°	1.19	0.12		45.8	39.3

Certain exceptions to the sudden vasoconstriction should be noted. First, the skin temperature did not always fall abruptly enough to produce a distinct 'angle', but in roughly one quarter of the experiments fell with increasing rapidity over a few degrees (indicated by a range of temperatures in the tables). Second, in six experiments (not included in the tables) there was a continuously gradual decline in skin temperature, rather than a sudden fall. This latter was found to be attributable in all but two of these experiments to the type of operation performed, or to the interval after operation, as described below.

III. *Degree of Vasoconstriction and Relative Decrease in Blood Flow*

1) *Burton's Thermal Conductivity Index.* Burton's (10) 'Thermal Conductivity Index' ('TCI') consists of the following ratio: $(T \text{ skin} - T \text{ air})/(T \text{ rectum} - T \text{ skin})$

which provides an approximate measure of peripheral blood flow provided the skin temperature has reached a steady state, having leveled off under conditions of relatively constant blood flow, rectal and air temperatures. As indicated in the tables, TCI of the operated paw was calculated for each experiment at the 'angle' in the skin temperature graph (before vasoconstriction) and again after the skin temperature had leveled off at its lowest value (after vasoconstriction). Comparison of averages

TABLE 2. SERIES II. CATS LIGHTLY ANESTHETIZED WITH NEMBUTAL—COLD ROOM

ANIMAL NO.	OPERATIVE PROCEDURE	DAYS POST-OP.	TEMP. AT TIME OF SUDDEN VASOCONS., °C.			THERMAL CONDUCTIVITY INDEX		STRAIGHT-NESS OF SEMI-LOG PLOT	TIME CONSTANT k, MIN.	
			Room	Rectal	Skin, paw	Before vaso-cons.	After vaso-cons.		Normal paw	Op. paw
3	Section sciatic	0.2	5°		*Paw cooled to 23° without vasoconstriction*				23	
		2	5°	33°	?26° (fan used)	2.5				
		5	17°	39.8°	29.6°	1.1	0.05	++++		24
		7	18°	37.4°	28°	1.0		+++	21	22
15	Section sciatic	2	4-2°	30.4°	19°	1.2			22	28
		4	5°	38°	29.1	2.7				
		6	13°	37.8°	27°					
		9	20-18°	37.8°	30.7°	1.3	0.22	+++		19
17	Bilat. lumbar sympathec-tomy & de-nerv. adrenals	7	11°	32.8°	L. 23	0.93	0.10	++++	19	19
					R. 22	1.5	0.10	+++	20	25
17	"	38	16°	31.5°	L. 22-23	1.0	0.11	++++	22	23
					R. 23	1.2	0.16	++	22	33
9	Section sciatic Adrenalectomy	40	21°	39°	29.7°	0.96	0.07	++		30
21	Total denerv. leg Adrenalectomy	30	17°	37.7°	26-27	1.1	0.16	++++	21	17
18	Total denerv. leg	5	14°	32.2	25-27	2.0	0.12	+++	16	32
20	Total denerv. leg	3	16°	39°	22-26	0.77	0.05	+++	23	24
Averages						1.38	0.11		20.9	24.7

of these two values indicates that the blood flow decreased by a factor of 9.9 in the dog and 10.5 in the cats (fig. 3, *lower*).

    2) Semi-logarithmic plots of declining portions of skin temperature curves.   *a)* *Straightness of plotted curve.* When the declining portions of skin temperature curves such as those in figures 1 and 2 were plotted semi-logarithmically, the resulting curves were found in most cases to be straight lines, which indicates that the original skin temperature graph was an exponential curve of the form $(T - T_o) = e - t/k$ where T = skin temperature, T_o = minimal skin temp., t = time, and k is the 'time con-

TABLE 3. SERIES III. CATS LIGHTLY ANESTHETIZED WITH NEMBUTAL—PAWS IN COLD BOX

| ANI-MAL NO. | OPERATIVE PROCEDURE | DAYS POST-OP. | TEMP. AT TIME OF SUDDEN VASOCONS., °C. | | | THERMAL CONDUCTIVITY INDEX | | STRAIGHT-NESS OF SEMI-LOG PLOT | TIME CONSTANT k, MIN. | |
			Room Box	Rectal	Skin, paw	Before vaso-cons.	After vaso-cons.		Nor-mal paw	Op. paw
17	Bilat. lumbar sympathect. and denerv. adrenals	52	29° —— 17°	38°	L. 26–29° R. 29–31°	1.2 2.3	0.12 0.25	++	71 17	17
24	L. lumbar sympathect., r. adrenal denerv., l. removed	14	24° —— 17°	38°	30.4° rose at 20.4°	1.5	0.27	++	26	20
27	L. lumbar sympathect., r. adrenal denerv., l. removed	11	30° —— 15°	38.6°	28–29°	1.65	0.03	++	24	20
23	Leg denervated Adrenalectomy	7 0	32° —— 0°	41.5°	22.3–24°	1.4	0.16	+++		16
28	Total denervation leg, including section of skin of thigh	6	31° —— 15°	36°	27.8°	2.1	0.13	+++	16	17
30	Leg denervated Leg amputated, except art. & vein	4 0	34° —— 9°	39.2°	26.2° rose 22.6–24.6°	1.2	0.19	++++	16	17
25	Section sciatic[1]	14	30° —— 0.5°	41.5°	23–26°	1.7	0.08			
25	"	7	30.5° —— 14°	34°	26°	2.2	0.18	+++	20	18
25	"	21	30° —— 11°	34.5°	23.5°	1.1	0.16	+++	23	15
24	Complete section of brachial plexus	14	23° —— 1°	37°	28–29° rose at 19°	3.1	0.4			
26	Section sciatic	3	32° —— 1°	39.5°	20	0.93	0.27	++		21
22	"	4	32° —— 2°	37°	25–27°	2.6	0.32	+++		17
Averages...............................						1.78	0.19		20	17.8

[1] The sciatic nerve was sectioned following evidence for regrowth of the one lumbar sympathetic chain which had been removed previously.

stant' of the curve. Figure 3, *upper right*, shows one of these plots from an experiment on a cat with a denervated leg. As indicated in the tables, the straightness of each curve was graded on an arbitrary scale of 0 to 4 plus.

Newton's 'Empirical Law of Cooling' predicts that the temperature of a given warm body with constant thermal conductivity will decline along a curve having an exponential equation of this type. Conversely, if a declining temperature curve is found to have an equation of this type, the thermal conductivity of the corresponding warm body must be constant. In the paw this indicates that the blood flow was
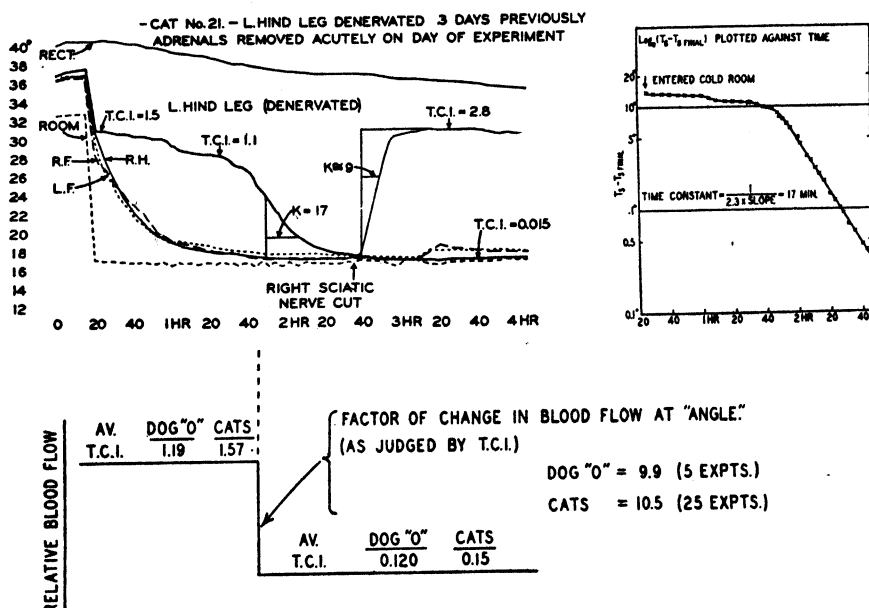
Fig. 3. *Upper left* shows sudden vasoconstriction after adrenalectomy; also illustrates method of determining, by direct measurement on the curve, the time constant 'k' (an indirect measure of relative blood flow) of an exponentially declining, or rising skin temperature curve. Thermal Conductivity Indices were calculated at points shown. *Upper right* illustrates straightness of semi-log plot of declining skin temperature curve, and more accurate method of determining k from slope of semi-log plot. *Lower* summarizes results of analyses of skin temperature data from 5 experiments on a dog, and 25 experiments on cats. Coincident with the 'angle' in a skin temperature graph, as in the upper left of this figure, there was, as a result of sudden vasoconstriction, a sudden decrease in relative blood flow, to an average value of approximately $\frac{1}{10}$ the previous value.

constant throughout the declining portion of the curve, because the thermal conductivity of the paw is made up of *a)* the thermal conductivity of the dead tissues (constant) plus *b)* the blood flow.

Therefore, the decrease in blood flow, which fell to approximately one tenth of its previous value, as measured by the thermal conductivity index, occurred suddenly at the time of the 'angle' in the skin temperature graph, and no further decrease occurred thereafter. These facts are summarized diagrammatically in figure 3, *lower*.

     b) Thermal time constant 'k'. The reciprocal of the slope of the semi-logarithmic

plot gives the thermal time constant, '*k*' in minutes, which may also be determined, though less accurately, by measuring on the original skin temperature graph the time required for the curve (if on inspection it appears to be an exponential) to decline to 36.8 per cent of its original height, or to rise to 63.2 per cent of its final height, as shown in figure 3, *upper left*. From mathematical considerations, Burton (11) has shown that there should be a linear relation between $1/k$ and the Thermal Conductivity Index. Thus, in a given paw k, as well as TCI, provides an approximate measure of relative blood flow, a short k corresponding to a rapid flow.

As shown in the tables, the values of k for the operated paws are of the same order of magnitude as for the normal paws, which indicates that vasoconstriction as a result of cold reduces the blood flow in the operated paw by approximately the same amount as reflex vasoconstriction does in the normal. That the blood flow was extremely small in either case is shown by the fact that when animals were killed while their paws were vasodilated, the values of the resulting k's were not significantly greater than for the vasoconstricted paws of living animals.

IV. *Rise in Critical Temperature with Increasing Intervals after Denervation*

Reference to table 1 indicates the relative constancy of the critical temperature for the sympathectomized paw or paws of the dog, the individual values varying by no more than $\pm 2°C$. from the average value of $22.1°C$. in all but one out of six values, during a period of from 10 to 130 days after lumbar sympathectomy   In view of the progressive 'regain of tone' known to occur in blood vessels during the first few hours or days after they have been deprived of their sympathetic innervation, it was decided to determine whether there was a day-by-day rise in the value of the critical temperature, indicating increasing 'sensitivity' to cold during the period immediately after operation.

Though they were attempted earlier, successful experiments on a dog less than 7 to 10 days after lumbar sympathectomy were found to be impossible owing to the period required for convalescence. It was therefore decided to section the sciatic nerve, containing the post-ganglionic sympathetic nerves to the paw, an operation so trivial as to require no period of convalescence. As shown in table 2, a series of experiments was performed on each of 2 cats (no. *3* and *15*) at varying intervals after sectioning the nerve. The results indicate that there was a definite tendency for the critical temperature to rise during the first few days after this operation. Thus, five hours after cutting the sciatic nerve in *animal 3*, there was no sudden vasoconstriction, the paw remaining vasodilated even though it was cooled to 23°C., by reducing the temperature of the room to 5°C. However, two days after operation, sudden vasoconstriction occurred when the skin was slightly below 26°C., the exact value being obscured by the brief use of a fan to cool the paw. In two experiments performed on the same animal on the fifth day, and in another experiment on the seventh day after operation, the critical temperature ranged between 28° and 29.6°C , with no further rise occurring after the fifth day. Very similar results were obtained in the experiments on *animal 15*, no further rise in critical temperature occurring later than the fourth day after operation.

When skin temperature studies were made on animals at intervals greater than

two, or three weeks after sectioning the sciatic, or all nerves to the leg, there was found to be a decreasing tendency for the corresponding paw to remain vasodilated and warm. This is in keeping with the observations of Goltz in 1874 and of others (4, 5). Thus, when the animal entered a room which was only moderately cold, the skin of the operated paw often declined in temperature as soon as the normally innervated paws, or its temperature 'floated', gradually shifting up and down at levels somewhat above those of the normally innervated paws. This behavior of the denervated paw is in contrast to the vasodilatation which persisted at moderately low room temperatures, i.e. not cold enough to cool the paw below its 'critical temperature', for weeks or months after (preganglionic) lumbar sympathectomy in the dog we have studied and in the one similarly operated cat followed for a prolonged period.

V. Exclusion of Certain Possible Causes of the Sudden Vasoconstriction

a) *Adrenal medulla.* That epinephrine may contribute to the vasoconstriction which occurs in blood vessels following severance of the sympathetic nerve fibers, with resulting sensitization to the hormone, has been emphasized by Smithwick (12) and others. Moreover, Elliott (13) and Ascroft (6) have suggested that cold potentiates the action of epinephrine, inasmuch as a given dose of epinephrine will produce a greater drop in skin temperature in a cool extremity than in the same extremity tested while warm. For these reasons it was of primary importance to determine whether sudden vasoconstriction might occur following denervation or removal of the adrenal glands.

In five experiments, both adrenals were denervated previously, or one adrenal was removed and the other denervated (*animals 17, 24, 27*, tables 2 and 3). In three experiments adrenalectomy was performed on the day of the experiment (*animals 9 and 21*, table 2, *animal 23*, table 3). When each of the former group of animals was autopsied, the extent of denervation was found to be almost but not wholly complete, one fine nerve twig running to the adrenal or adrenals from the remaining upper portions of the lumbar sympathetic chains. (The second experiment on *animal 24* was not included in the denervated group because a time sufficient for regeneration had elapsed.) In each of these eight experiments, with the adrenals (almost) totally denervated or removed, there was a fall in the skin temperature of the operated paw which was no less sudden than in the animals with intact adrenals. One of these records, from *cat 21*, Series II, is shown in figure 3, *upper left*. The sudden rise in the temperature of the normal paw following sectioning of the corresponding sciatic nerve provides a check on the adequacy of the blood pressure and the absence of shock.

b) *Possible persistence of nerves to blood vessels.* With the exception of one experiment, in which the skin temperature declined gradually, there was no essential difference between experiments with animals in which the sciatic nerve alone was cut, and experiments with 6 animals, (nos. *18, 20, 21*, table 2, and nos. *23, 28, 30*, table 3) with total denervation of the leg. The sciatic nerve, femoral nerve and a branch of the obturator nerve were cut and, in addition, in three of these animals the skin and subcutaneous tissues of the leg were cut completely around in the thigh and then sewed up. Portions of the femoral artery and vein were dissected free and soaked

several times with 10 per cent iodine or 95 per cent alcohol. As a final check, in one experiment, (*animal 30*, table 3) four days following section of the nerves to the leg, the leg was completely amputated in mid-thigh, including all tissues and the bone, except for the artery and vein. These vessels were dissected free and were wrapped in cotton soaked with 2 per cent procaine. The bone was wired together and the skin sewed up. As shown in figure 4, sudden vasoconstriction occurred on cooling the box and sudden vasodilatation occurred on subsequently warming the box.

c) *Humoral agents other than epinephrine.* The tendency of a cold environment to cause the body to produce a circulating vasoconstrictor substance was reduced to a minimum in one experiment (the first of 3 on *animal 25*, table 3) by applying heat by means of an electric pad to the body of a cat located in a room at 30°C., whose hind paws, projecting into the cold box, were the only parts of the animal exposed to cold.
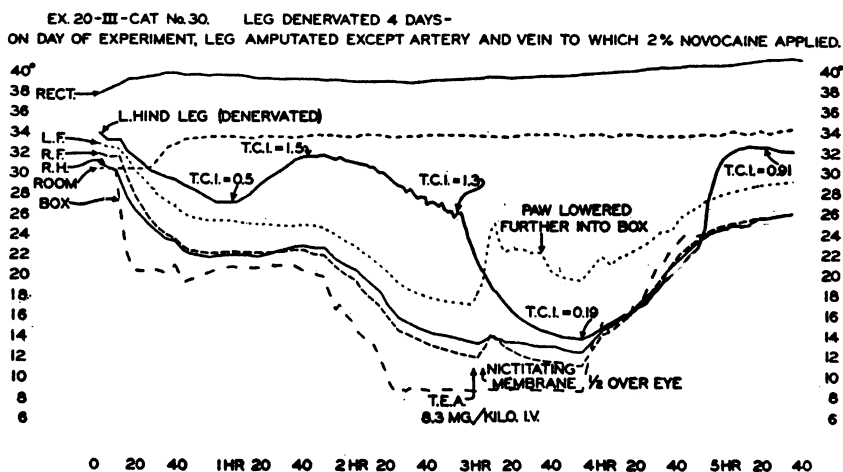
Fig. 4. SUDDEN VASOCONSTRICTION, and later sudden vasodilatation, occurring after exclusion of possible persisting nerves by amputation of leg, with the exception of artery and vein, which were procainized. Both hind paws were alone cooled, but portions of legs above paws were kept warm. Sudden vasoconstriction occurred in previously denervated paw after cooling air in box, and later in the experiment sudden vasodilatation occurred in paw following warming of box.

The chart is shown in figure 5. At the time when the operated paw (sciatic nerve sectioned) showed the characteristic sudden vasoconstriction as a result of cold, the normally innervated paw was warm, indicating absence of reflex vasoconstriction. At the time of the arrow on the chart, the heater pad was turned off, following which the normal paw constricted reflexly as shown. This experiment tends to rule out sympathin as causing the vasoconstriction, inasmuch as generalized sympathetic activity as a result of cold was eliminated by keeping the animal warm. Similarly, the experiment tends to rule out other possible vasoconstrictor substances which might be released as a result of exposure of the animal to cold.

An additional factor tending to rule out sympathin or another vasoconstrictor agent is the sudden vasodilatation occurring in the denervated or sympathectomized limb when the air was warmed, as shown in the latter part of the experiment charted

in figure 4.   In the three experiments in which this occurred the normal paw remained reflexly constricted, indicating generalized activity of the sympathetic nervous system in response to cold, yet the sympathin which must have been produced by this activity was incapable of preventing vasodilatation of the operated paw.

Attempts were made to induce the sudden vasoconstriction by injecting various substances or carrying out certain procedures when it was estimated that the skin of the operated paw was close to the 'critical temperature' for sudden vasoconstriction. All such attempts failed to produce the characteristic sudden fall in temperature. The agents and procedures used, with their effects on the skin temperature of the operated paw, were as follows: acetylcholine, 1 to 100,000, sodium bicarbonate, 2 per cent, both I.V., and CO_2 7.5 and 10.0 per cent, via tracheal cannula (slight rise in skin temperature), ammonium chloride, 2 per cent, I.V., and hyperventilation by means of artificial respiration apparatus, (slight fall in skin temperature).
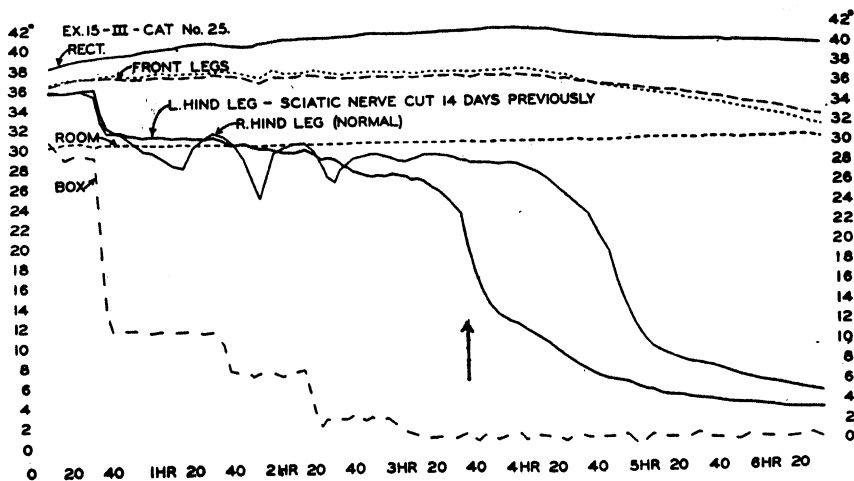
Fig. 5. SUDDEN VASOCONSTRICTION occurring in denervated left hind paw of animal kept warm enough to maintain reflex vasodilatation in normally innervated right hind paw.   At the arrow, the heater pad on the animal's body was turned off.

During the course of the investigation, it was found that pentobarbital, when administered in small maintenance doses (approximately 7 mg/kg. intraperitoneally), at a time which happened to coincide with the start of a sudden fall in skin temperature of the operated paw, often actually interrupted the fall, causing the skin temperature to rise again to its previous value for periods of varying duration.   In most instances the dose used was insufficient to abolish reflex vasoconstriction.   A similar interruption of the sudden fall in skin temperature was produced by means of a moderate dose (7 mg/kg. I.V.) of tetra-ethyl-ammonium bromide ('TEA') administered to an animal with section of all nerves of the brachial plexus (no. *24*, table 3). However, in the experiment in which the leg was amputated (fig. 4) there was no interruption of the sudden fall when TEA was injected.   These limited data suggest that TEA when it did produce vasodilatation in the operated paw may have done so by preventing impulses from passing down sympathetic nerves persisting in spite of

attempts to eliminate them, rather than via a direct vasodilating action on the blood vessels themselves, especially since TEA has no such vasodilating action when injected intraarterially into normal limbs (14).

DISCUSSION

Other factors having been excluded, it is our impression that the sudden vasoconstriction described here may be induced by the combined action of two local processes, as follows: first, as a result of denervation, the smooth muscle of the arteriole may become sensitized to cold, as suggested by Cannon (15). A second factor tending to reduce the blood flow, though not suddenly, may result from a reduced formation of vasodilator metabolites owing to cooling of the tissues, as described by Freeman (18).

As a partial test of the first hypothesis we (16) have studied the effects of cold on the normal and denervated nictitating membrane, a smooth muscle which Bozler (17) places in the same classification ('multi-unit') as the blood vessels, because of certain characteristics in common to both. The nictitating membranes (n.m.) were cooled by means of small glass bulbs which were inserted in place of the eyes of the cats. The effect of cold alone was tested first, by gradually lowering the temperature of water which was pumped through the interior of the bulbs and second, by suddenly lowering the temperature of the water from 37°C. by varying amounts. In two out of four experiments the chronically denervated n.m. showed true sensitization to cold, contracting in response to a smaller drop in temperature than the normal n.m., and showing a greater contraction than the normal with a given drop in temperature for all less-than-maximal contractions. In addition, the contraction of the denervated n.m. in response to cold was always jerky and sudden, producing a stepwise tracing, in contrast to the smooth continuous contraction of the normal n.m.

One would have at least a partial explanation for the sudden vasoconstriction should the chronically denervated blood vessel respond to cooling in the same way as the nictitating membrane. That certain types of blood vessels can become sensitive to local cooling is indicated by the observations of Lewis and Landis (19) in Raynaud's disease, in which arterial vasoconstriction occurred upon exposure to cold even after nerve block or sympathectomy. The fact that sudden vasoconstriction occurred in our experiments after cooling the paw alone, while keeping the leg warm, merely indicates that the response occurred either in the arteries of the paw itself or in the arteriovenous anastomoses of the skin of the paw, but does not distinguish between the two.

SUMMARY

A sudden, late fall of skin temperature was observed when denervated or sympathectomized paws of one unanesthetized dog and 16 lightly anesthetized cats were exposed to cold for prolonged periods. A corresponding abrupt rise of skin temperature was observed when the chilled extremities were exposed again to warm air. Analysis of the curves of skin temperature indicated that the vasoconstriction producing this sudden fall in skin temperature was marked enough to reduce blood flow to approximately one-tenth its previous value. The vasoconstriction appeared when

the skin of the operated paws reached a 'critical temperature'.   After preganglionic lumbar sympathectomy in the dog, this 'critical temperature' remained constant at approximately $22° \pm 2°C.$, up to 130 days postoperative.   After section of the sciatic nerve in cats, the 'critical temperature' ranged between 19° and 26°C. up to 2 days after operation and then rose to reach between 28 and 31°C. by 4 to 21 days post-operative.   This effect of prolonged cooling appears to be due chiefly to locally increased sensitivity of the denervated blood vessels to cold because the reaction appeared at the usual 'critical temperature' a) when the adrenal glands were extirpated or inactive, b) when the leg was disconnected from the body except for the procainized artery and vein and c) when only the paws were chilled while the animal's body was kept warm enough to produce maximal vasodilatation in the normally innervated paws.

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EFFECTS OF COOLING ON NERVE CONDUCTION IN A HIBERNATOR (GOLDEN HAMSTER) AND NON-HIBERNATOR (ALBINO RAT)

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L ITTLE attention appears to have been paid in recent years to neurophysiological differences between mammals capable of hibernation and those that are not. Horvath (1) pointed out that certain species (ground squirrel, hedgehog, European hamster, and bat) could be cooled artificially until their rectal temperatures approached 0°C. and still revive spontaneously on being returned to a warmer environment. On the other hand cooling to a rectal temperature of 10° to 19°C. is usually fatal to mammals which do not hibernate, for example, rabbits, cats, dogs, guinea pigs, rats and monkeys (2–8), although mice have survived a body temperature of 8.5°C. (9) and newborn rats have recovered from 5°C. (10).

Interest was aroused in the present problem when it was noted that hibernating golden hamsters with body temperatures of 4°C. as measured by rectal and cheek pouch thermocouples still responded to external stimuli. Since it had been shown that nerves of the cat studied *in vitro* cease functioning at about 8°C. (11), it was thought advisable to compare the effects of cooling on conduction by nerve in the hamster and a non-hibernating rodent, the albino rat.

METHOD

Adult golden hamsters (*Mesocricetus auratus*) and adult albino rats were killed by stunning or decapitation, the tibial nerves removed and placed in Ringer's solution at room temperature. Each of the pair of nerves was then in turn arranged for monophasic recording on silver-silver chloride electrodes in a double-walled moist chamber, the temperature inside of which could be changed in steps from 20°C. to 2°C. by means of circulating brine. The nerve was crushed under the distal recording electrode, but no attempt was made to minimize the positive artefact with KCl or cocaine. A thermometer with bulb close to the nerve measured the temperature inside the chamber. Complete observations were made on 10 nerves from hamsters, 3 of which were from hamsters which had been in hibernation for 32 to 52 days and on 12 nerves from rats.

Preliminary experiments showed that it took about 10 minutes for a nerve to reach equilibrium at any one temperature. Hence the nerves were always left at any given temperature for at least 10 minutes before observations were made.

The nerves were stimulated with supramaximal biphasic shocks from a Grass stimulator led to the nerve in the chamber through a Wagner ground. Nerve action potentials were led into a capacity-coupled amplifier on push-pull and thence to a cathode ray oscilloscope, where the potentials were either photographed or measured directly with a celluloid grid.

Since the nerves were short, especially those from the hamsters, we were able to obtain only one elevation of the compound nerve action potential. The variables measured were: height of the action potential, conduction velocity, excitability, the temperature at which the nerve ceased to conduct and, in a few cases, the absolutely and relatively refractory periods. Excitability was measured as the reciprocal of the voltage necessary to just cause the appearance of the nerve action potential. Thus when 'excitability' is used below it refers to the excitability of the fibers with lowest threshold in the two groups of nerves.

All measurements of the above variables made at 20°C. were arbitrarily taken to be 100 per cent and changes in the variables were recorded both in absolute figures and as percentages of the values at 20°C.

Gasser (12) has pointed out that the internal resistance of nerve fibers changes with temperature, which has an effect on the magnitude of the recorded potential. In order to evaluate this source of error, the resistances of tibial nerves from a hamster and rat were measured on electrodes in the chamber with an A.C. bridge at 3000 c/sec. On cooling from 20°C. to 6°C. the nerves showed an average increase in resistance of 61 per cent and the rates of change of resistance were similar. This increase is not great enough to exert significant influence on the results reported below.

TABLE 1. TEMPERATURES AT WHICH THE TIBIAL NERVES OF HAMSTER AND RAT CEASED TO CONDUCT

HAMSTER NERVE	TEMPERATURE, °C.	RAT NERVE	TEMPERATURE, °C.
Exp. H_1	3.0	Exp. R_1	7.0
H_2	2.0	R_2	9.5
H_3	3.0	R_3	7.0
H_4	3.5	R_4	12.0
H_5	3.5	R_5	8.0
H_6	2.5	R_6	11.0
H_7	6.0	R_7	10.0
H_8	3.5	R_8	9.0
H_9	3.5	R_9	7.5
H_{10}	3.5	R_{10}	10.0
		R_{11}	8.5
		R_{12}	8.5
Average.............	3.4		9.0

RESULTS

Minimal temperatures permitting conduction in nerves of hamster and rat. The most striking difference between the tibial nerves of the two species studied is that nerves from hamsters as opposed to nerves from rats will conduct when cooled to lower temperatures. Table 1 shows the temperatures at which nerves in each single experiment ceased to conduct.

As can be seen in table 1, the average critical temperature for functioning of nerves was for the tibial of the hamster 3.4°C. and for the rat 9.0°C. Statistical analysis of the figures of table 1 according to the 't' test of significance shows that P is much less than 0.01, hence the observations are statistically valid.

Effect of cooling on height of action potential, conduction velocity, and excitability. The differences in these respects between the nerves of hamster and rat can best be appreciated by reference to figures 1 to 3, which show typical results. In figure 1 it can be seen that cooling the tibial nerve of a rat (*exp. R10*) from 20°C. causes a progressive decrease in the height of the action potential and in conduction velocity. In figure 3 the relative changes in these variables for the rat are plotted, together with
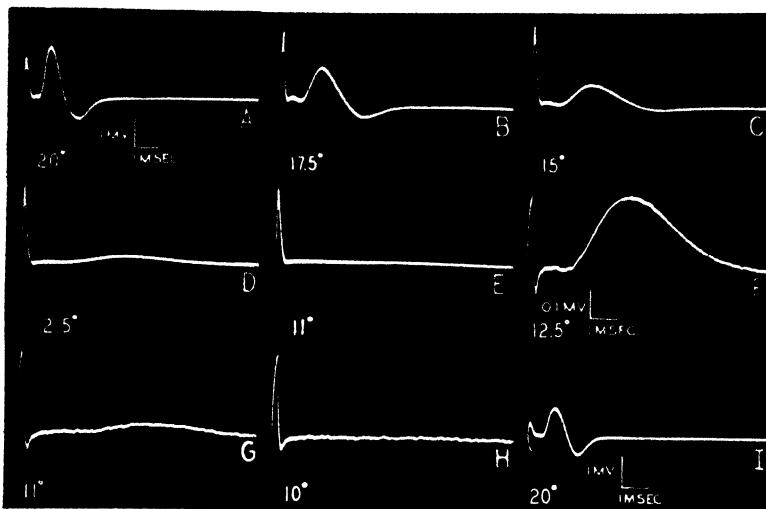
Fig. 1. ACTION POTENTIALS OF TIBIAL NERVE of a rat at various temperatures. *A* through *E* shows gradual decrease in ht. of action potential and conduction velocity on cooling, all at same gain. In *F*, *G* and *H*, with gain increased approximately 10 times, the nerve was cooled from 12.5°C. and the action potential was seen to disappear completely at 10°C. *I*, at original gain, shows the action potential when the nerve had been rewarmed to 20°C. (exp. *R10*).
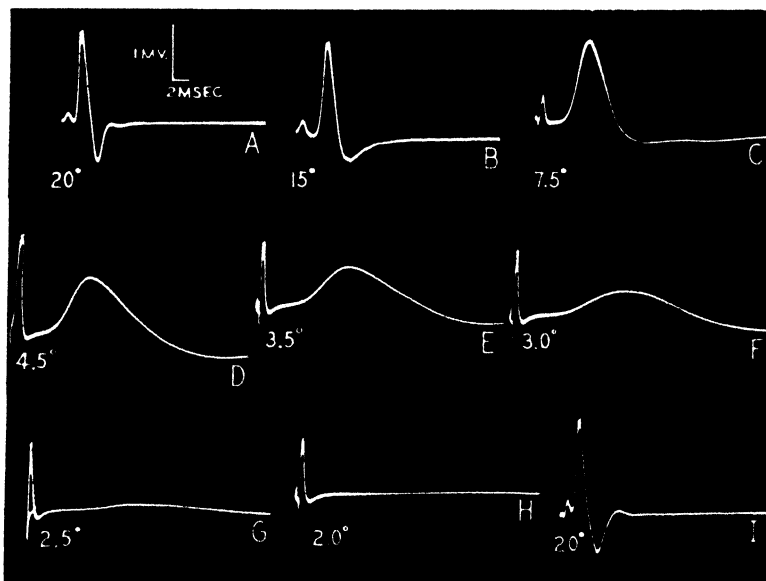
Fig. 2. ACTION POTENTIALS OF TIBIAL NERVE of a hamster at various temperatures. All potentials at same gain. *B* through *H*, gradual decrease in ht. of action potential and conduction velocity on cooling. Note that a small potential was still present at 2.5°C. (confirmed with higher gain). *I* shows the potential when the nerve had been rewarmed to 20°C. (exp. *H2*).

the change in the excitability of the nerve. All three variables are seen to decrease fairly linearly and at approximately the same rate.

Figure 2 shows the action potential of a typical nerve from a hamster (*exp. H2*) which in this case happened to be from a hibernating hamster, although nerves from non-hibernating hamsters behaved similarly. Figure 3 reveals that while again in the hamster there is a fairly linear decrease in conduction velocity and excitability with cooling, the curve is shifted markedly to the left from that of the rat and there is a dissociation in that the action potential actually increases in height transiently as cooling progresses.
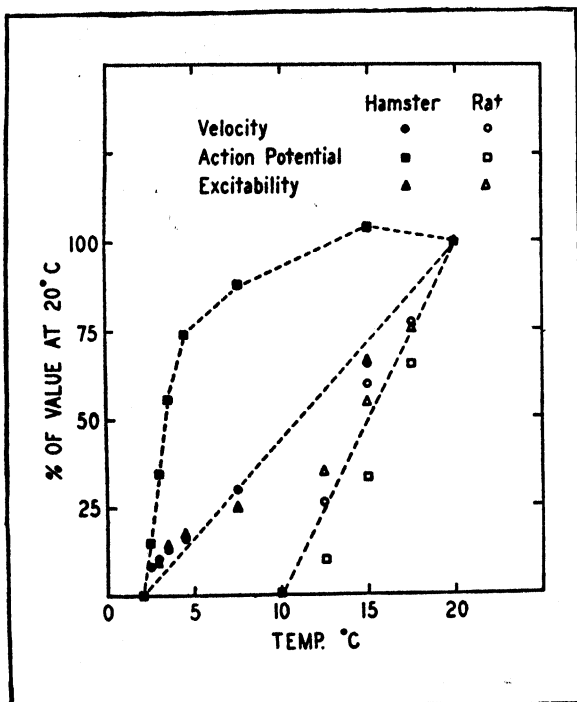
Fig. 3. PLOT OF CONDUCTION VELOCITIES, action potentials, and excitabilities of the nerves of figs. 1 and 2 at various temperatures, showing the fairly linear decrease in these 3 variables on cooling for the rat, the shifted curve of decrease in conduction velocity and excitability for the hamster and the transient increase in ht. of action potential found in the hamster.

Figures 1 and 2 illustrate one other difference between nerves of hamster and rat, namely, that when the nerves were rewarmed to 20°C. after they had been cooled till conduction ceased, nerves from rats in only 2 out of 12 experiments showed the original height of the action potential, while the nerves of the hamsters in all but one instance showed their original potential or even exceeded it.

Effect of cooling on absolutely and relatively refractory periods. These have not been analyzed with precision, but we can state that, as might be expected, cooling increased the duration of these refractory periods. Furthermore, nerves from hamsters required a greater amount of cooling than nerves from rats to bring about the same relative changes in refractory periods.

Fatigue in cooled nerve of the hamster. We have noticed in nerves of hamsters that, at temperatures of 5°C., repetitive stimulation at rates as low as 1/sec. resulted

in rapid fatigue as evidenced by a progressive decrease in the size of the action potential.

Absolute conduction velocities in nerves from hamsters and rats. Conduction velocities in nerves of hamsters at 20°C. averaged 30.5 m/sec., while nerves from rats at the same temperature conducted at an average rate of 37.8 m/sec. Statistical analysis showed no significant difference between the two groups. Measurement of conduction velocity in such short lengths of nerve is difficult to do accurately, but the highest figure obtained for the rat (69.2 m/sec.) is of the order of magnitude one would expect from consideration of the known maximal fiber diameter in the tibial nerve of this species (13).

Similarity of function in nerves from hibernating and non-hibernating hamsters. When it was discovered that nerves from hibernating hamsters conducted at lower temperatures than those from rats, the question arose as to whether the resistance to cold was a property which the nerves acquired as the hamster went into hibernation or whether this property was intrinsic to the species. Subsequent studies revealed no differences in thermal sensitivity between the nerves of non-hibernating and hibernating hamsters. Thus the property of being able to conduct at low temperature is a true species difference and not a change in the nerve which occurs when the hamster is exposed to cold.

<center>DISCUSSION</center>

The observations reported here would appear to confirm the dictum of Horvath (1) that "artificial cooling has clearly shown that hibernators react to cooling completely differently than non-hibernators." Indeed Tait (14) stated that a phrenic nerve-diaphragm preparation, as well as the excised heart, from hibernating animals (woodchuck, hedgehog) showed activity at much lower temperatures than would be expected were the preparations from non-hibernating animals.

The fact that the action potential and conduction velocity of mammalian nerve decrease with cooling has been recorded by Gasser (12). If this decrease is assumed to be linear, an extrapolation made of Gasser's figures for the phrenic nerve of the dog shows that this preparation should cease to conduct at about 11°C., which is comparable to our results with the tibial nerve of the rat.

When nerves from hamsters are cooled, conduction velocity and excitability seem to decrease at the same rate, while in the early stages of cooling the height of the action potential is well maintained or may even increase. The literature on the effects of cooling on the height of the action potential is controversial. For example, Gasser (12, 15) described a decline in height on cooling in mammalian and frog nerves, whereas Schoepfle and Erlanger (16) found that cold increased the height of the action potential in frog single-fiber preparations and Lundberg (17) found that cooling increased the height in mammalian *C* but not mammalian *A* fibers. At any rate, the behavior of nerve from the hamster on cooling shows a dissociation between height of the action potential and conduction velocity which, if examined in the light of current theories of nerve conduction, indicates that the usual proportionality between these two variables may be more fortuitous than fundamental.

The hamster shows a resistance and adaptation to cold which the rat does not

possess. The mechanism of the differences in response to cooling displayed by nerves from the two species remains obscure. Lundberg (17) has recently shown that C fibers are more resistant to cooling than A fibers. However, it is doubtful that the physiological differences between nerves from hamsters and rats could be based merely on differences in fiber size, since at low temperatures the nerve of the hamster is more excitable and has a larger action potential than one would expect if the still active fibers were only the smallest ones.

Studies on the metabolism of hibernating hamsters in a cold room (4°C.) with a body temperature of 4°C. have shown (18) that, if the room temperature is dropped another 2° to 6°C., the animal's temperature will again begin to decline. Under these circumstances one of three things may happen—the animal's temperature may drop until it dies, the hibernator may 'wake up'[3] or it may remain in hibernation but increase its oxygen consumption so that its body temperature is maintained at 2.5°C. —that is, above the new temperature of the cold room. It is significant that this figure for maintained body temperature is just above the lowest critical temperature for nerve functioning which we have found (2°C. in a hibernator). Hence the hamster shows remarkable adaptation to low temperatures both by a reflex metabolic adjustment for survival and by a tolerance of its nerves for cold not shown by the non-hibernator.

SUMMARY

The golden hamster (*Mesocricetus auratus*) hibernates when exposed to cold whereas the rat does not. The behavior of tibial nerves from these two species was studied during cooling to determine whether the effects on the height of the action potential, conduction velocity, excitability and refractory periods would demonstrate species differences in the resistance of nerve to cold.

Nerves from hamsters did not cease functioning until an average temperature of 3.4°C. was reached, while nerves from rats ceased functioning at an average temperature of 9°C. When nerves from rats were cooled, the action potential, conduction velocity and excitability decreased linearly with temperature. When nerves from hamsters were cooled similarly these variables decreased at a slower rate. The action potentials increased in amplitude in the early stages of cooling and then declined.

Cooling increased the duration of the absolutely and relatively refractory periods of nerves from both animals, although relatively less so in the hamster than rat. Tibial nerves of hamsters, though capable of functioning at low temperatures, fatigued rapidly at such temperatures. The critical temperature at which peripheral nerve of the hamster ceases to function is at a level just below that at which hibernating hamsters have been found to maintain their body temperatures by metabolic means when exposed to extreme cold.

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[3] We use the words 'wake up' advisedly. As Horvath (1) said, "Fassen wir alle unsere Kenntnisse über den Winterschlaf zusammen und ziehen besonders den sommerlichen Winterschlaf und unsere volle Unkenntniss des gewöhnlichen Schlafes in Betracht, so gelangen wir immer mehr und mehr zur Einsicht, mit wie viel Recht man (jetzt noch) sagen kann; 'Der Winterschlaf ist erstens kein Schlaf, und zweitens hat er gar nichts mit dem Winter zu thun' ".

The results reported are regarded as evidence of an intrinsic adaptation to cold possessed by a species capable of hibernation.

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HISTAMINE AS THE POSSIBLE CHEMICAL MEDIATOR FOR CUTANEOUS PAIN

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PREVIOUS studies (1–5) have indicated that histamine, or a histamine-like substance, may be a peripheral mediator of cutaneous pain. By use of sensitive biological methods, it was demonstrated that irritation of the skin or cornea by mechanical, electrical or chemical stimuli, below the threshold for injury, is associated with the liberation of histamine or a histamine-like substance; the quantity liberated varies directly with the intensity of the stimulus. In addition, the perfusion of a solution of histamine onto the denuded skin or its intracutaneous injection is associated with painful sensations.

As a corollary, it was shown that certain histamine antagonists (phenol ethers), in sufficient subcutaneous dosage, produce a generalized peripheral anesthesia in the dog, monkey and human being; on intracutaneous injection, they produce local anesthesia (6). It has since been reported that other anti-histamine drugs, of widely varying structure, likewise act as local anesthetics (7).

A recent study by V. Euler (8) has shown the presence of a relatively high concentration of histamine-like substance in mammalian sensory nerve: 25 to 40 $\mu g/gm$. of nerve. By comparison, only 0.01 μg. of acetylcholine and 1 μg. of sympathin (dS—noradrenaline) per gram were found. Here, again, a rôle of histamine in mediation of pain is suggested.

The present report concerns itself with the determination of the minimum concentration of histamine necessary to produce sensations on intradermal injection. As a control, the actions of acetylcholine, potassium chloride and adenosine, substances which might conceivably play a rôle in pain mediation, were also tested, alone and in combination with histamine.

METHOD

Twenty-seven adult (male and female) subjects were tested: Caucasian, Negro and Mongoloid racial types were represented in the series. The subjects were members of the faculty of the University of Illinois and, to a lesser extent, of the student body of the College of Medicine.

All solutions used for injection were made up fresh each day, in chemically clean and sterile glassware. The diluent for all test substances was a sterile (pyrogen-free or not) solution of 0.85 per cent NaCl in distilled water, always taken from the flask containing the saline used for the control (blank) injections. Histamine, as the dihydrochloride, and acetylcholine, as the chloride, were

diluted directly from sterile ampules. Potassium chloride and adenosine solutions were autoclaved at 15 pounds pressure for 15 minutes.

At the start of each experiment, the subject was put at rest in a comfortable reclining chair and one arm, the volar surface cleansed with soap, water and alcohol, was held in the lap of the experimenter. The subject was not told of the nature of the experiment, other than that there would be a series of intradermal injections, and was not allowed to look at the arm throughout the experiment. He was instructed to state immediately the occurrence of any sensation following the particular injection; this was noted and timed with a stopwatch.

For the injection, 27-gauge needles and 2-cc. syringes were used; a different set was employed for each concentration of the respective solutions. The needle was gently inserted, as superficially as possible, into the skin of the volar surface of the forearm. No injection was made until the pain from this prick had subsided. About 0.01 ml. was injected, producing an initial wheal of 2 to 3 mm. The injections were randomized with saline controls interspersed; there were as many saline placebo injections as of any given solution used. The results of each injection were timed for a minimum of three minutes.

<div align="center">RESULTS</div>

The accompanying table summarizes the essential results. It will be noted that definite painful sensations were reported with histamine at concentrations of 10^{-15} and 10^{-18}; the results were significant within the 5 per cent limit of confidence (P value 0.05). In 11 of the 44 trials at these two concentrations, a double sensation occurred, e.g. prickling became a dull pain or vice versa. On the contrary, when saline alone was used, no more than one sensation was experienced, if any, and this was of much shorter duration than that following histamine. This is illustrated by the following protocol of any experiment on a white male subject, using 10^{-15} histamine:

Secs. after injection	Sensation	Secs. after injection	Sensation
0	Nothing	100	Pain decreasing
30	Low intensity pain	120	Very slight pain still present
60	Increasing pain, same quality as above	150	No pain, but 'awareness' at site of injection
83	Pain still present	170	All sensation gone
90	Pain at maximum		

With the same individual, one response to saline was entirely negative and the other two were as follows:

Secs. after injection	Sensation	Secs. after injection	Sensation
0	Nothing	60	Nothing
15	Nothing	90	Nothing
30	Nothing	120	Nothing
45	Slight pain of very low intensity		

Generally, at the higher concentrations of histamine, 10^{-8} and greater, three types of sensations were reported with each injection.

Itching, usually following an initial pain, occurred with concentrations of 10^{-8} and greater, but not at the lower concentrations. Secondary wheals and flares

TABLE I.   RESPONSE TO HISTAMINE INJECTED INTRADERMALLY (27 NORMAL ADULTS)

CONCENTRATION[1]	×10^-5 (2.5 to 1.25)		×10^-6 (10 to 3.1)		10^-7 to 10^-8		10^-9 to 10^-11		10^-12 to 10^-14		10^-15		10^-18		Saline	
Type of sensation	Obs.[2]	Pos.[3]	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.
	10		10		54		11		26		24		20		60	
Prickling, stinging or tingling.																
As 1st sens.[5]		1		2		15		3		3		5		6		7
as 2d sens.		0		1		3		2		0		2		0		0
as 3d sens.		0		0		2		0		0		0		0		0
Total		1		3		20		5		3		7		6		7
Burning																
As 1st sens.		0		1		9		0		3		3		3		4
as 2d sens.		6		3		1		0		0		0		0		0
as 3d sens.		1		0		0		0		0		0		0		0
Total		7		4		10		0		3		3		3		4
Itching																
As 1st sens.		0		1		4		1		0		0		0		3
as 2d sens.		4		3		9		0		0		0		0		0
as 3d sens.		4		2		2		0		0		0		0		0
Total		8		6		15		1		0		0		0		3
Pain (dull)																
As 1st sens.		2		1		11		1		3		7		2		7
as 2d sens.		0		1		7		0		2		1		2		0
as 3d sens.		0		9		0		0		0		0		0		0
Total		2		11		18		1		5		8		4		7
Pain (sharp)																
As 1st sens.		7		5		9		2		2		3		2		0
as 2d sens.		0		1		6		0		1		5		1		0
as 3d sens.		0		0		0		0		0		0		0		0
Total		7		6		15		2		3		8		3		0
Grand total	10	10[4]	10	10	54	48	11	7	26	11	24	18	20	13	60	21
Percentage positive	100%		100%		89%		63%		42%		75%		65%		35%	
Value of p[6]	<0.01		<0.01		<0.01		<0.05		<0.05		<0.01		.05-.01			

[1] Concentrations are calculated for histamine base.

[2] Obs. = Number of observations (individual injections); [3] Pos. = Number of positive responses.

[4] Grand total of positives is sum of positive injections without regard to number of sensations produced.

[5] 1st, 2d and 3d sensations refer to order of appearance of respective sensations as reported by subject after each injection.

[6] p is calculated from chi-square of fourfold table, with grand total figures for each concentration of histamine compared with those for saline.

(triple response) were also absent with concentrations less than 10^{-8}; when present, they were not always accompanied by itching (9). No correlation in sensitivity to histamine was found witheither sex or race.

Potassium chloride alone at 1:100 caused a sharp stinging, while 1:1000 dilution gave no sensation. Acetylcholine at 1:500 produced a definite burning sensation but little to no sensation at 1:1000 dilution; when combined with histamine, at a final dilution of 1:1000, it did not augment the pain-producing action of histamine. A similar lack of augmentation was found with adenosine (1:2000 final dilution).

<div align="center">DISCUSSION</div>

By application of Avogadro's Number, it may be shown that 0.01 ml. of 10^{-18} histamine base contains 54 molecules of the active agent. Errors in the serial dilution may, of course, cause the actual number to vary by 100 to 200 per cent. Even so, since such an extremely small number of molecules is associated with definite painful sensation, the postulate of specificity of histamine (or a histamine-like substance) as an integral part of the peripheral cutaneous pain mechanism is strongly supported. The possibility remains that the physiologic substance is only 'histamine-like', and that the injected histamine simulates this substance. The decision on this point must remain in abeyance until more specific methods are available for characterizing the naturally occurring substance, released on painful stimulation.

Our results on the action of acetylcholine are in variance with those of Emmelin and Feldberg (10), who reported that this substance, injected into the skin by puncture with a sharp needle, at 1:50 and 1:100 caused no sensation, but in combination with histamine caused a burning sensation. The discrepancy in observed responses may be due to the difference in methods of injection.

Our results indicate that itching is not a 'subthreshold' pain, since production of an itch requires a stimulus much stronger than that for a pain. It appears, rather, that the sensation (s) variously described by our subjects as 'prickling', 'tingling', or 'stinging' is most likely the least perceptible manifestation of pain.

<div align="center">SUMMARY</div>

Painful sensations may be produced by the injection of histamine, at concentrations as low as 10^{-18}, into the superficial layers of the cutis. These findings are taken to indicate specificity of histamine in production of cutaneous pain and substantiate the postulate that a histamine-like substance acts as a physiologic mediator of pain. Acetylcholine and adenosine apparently do not augment the cutaneous pain-producing action of histamine. The fact that production of itching requires a higher concentration of histamine than that necessary for pain indicates that itching is not a 'subthreshold' pain. The least perceptible manifestations of pain are 'prickling', 'stinging' or 'tingling'.

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RELATION OF CEREBRAL ARTERIOVENOUS DIFFERENCE IN OXYGEN CONTENT TO ARTERIAL CARBON DIOXIDE TENSION

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L ENNOX, Gibbs and co-workers (1–3) have published a series of investigations leading to the theory that the brain is protected from rapid alteration of its acid-base balance by compensatory changes in the cerebral blood flow. This regulatory mechanism is considered to be functional only in the presence of adequate oxygen, the need for oxygen being paramount. The theory is based in large part on studies of cerebral arteriovenous differences as observed in human subjects, the cerebral venous blood being obtained from the internal jugular vein.

This theory was questioned by Schmidt and co-workers (4, 5), who measured the cerebral blood flow and gaseous metabolism in the monkey and concluded that the tone of the cerebral vessels tends to alter in accordance with the oxygen requirement of the brain, which varies with its functional activity. Gibbs, Maxwell and Gibbs (3) pointed out that the values for oxygen content of the blood appeared to be considerably below normal in the experiments of Schmidt and co-workers. Geiger and Magnes (6), using the perfused brain of the cat, obtained results which agreed in part with those of Schmidt and co-workers. They found that the cerebral blood flow could be correlated with oxygen consumption at the lower levels of blood flow, but not at the higher levels. Carbon dioxide showed only a slight regulatory effect on blood flow. In a study of the effects of hyperventilation on human subjects, however, Kety and Schmidt (7) reached conclusions which are in accord with those of Lennox and co-workers.

Some of the interpretations of Lennox, Gibbs and Gibbs and of Kety and Schmidt have been called into question by Ferris and his co-workers (8), who, while not disputing the regulatory function of carbon dioxide, found that variable amounts of extracranial blood may be included in specimens drawn from the internal jugular vein

In this paper are presented some measurements of cerebral A-V differences in oxygen content obtained on dogs. Blood samples were obtained from the superior sagittal sinus representing the blood draining the cerebral cortex without admixture of significant amounts of extracranial blood. These data have been obtained in the course of studies on metrazol convulsions (9) and on the relations between the blood gases and certain constituents of the cerebral tissue (10). The findings are in agreement with the views of Lennox and co-workers.

PROCEDURES

Dogs were given 20 mg. morphine sulfate/kg. body weight by subcutaneous injection. A Magill intratracheal tube with a Waters-Guedel inflatable cuff was inserted. Local infiltration with 0.25 per cent procaine hydrochloride was used for exposure of the skull and of the femoral arteries and veins. A trephine opening was made over the superior sagittal sinus and the bone of the calvarium was removed over a wide area, leaving the dura intact. One femoral artery was cannulated for the measurement of blood pressure. At this point the 'normal' blood specimens were drawn simultaneously from the femoral artery and the sagittal sinus, being collected anaerobically over mercury with heparin as the anticoagulant.

Mixtures of oxygen, carbon dioxide and nitrogen were administered from a 5-liter breathing bag connected to a Foregger anesthesia apparatus. During the period of administration of the desired mixture, 12 to 14 minutes after its inception, blood specimens were again taken simultaneously from the artery and the sagittal sinus.

Decreased arterial carbon dioxide tension was attained by hyperventilation with air or oxygen. In this group of experiments the animals were immobilized by intravenous injection of a 0.75 per cent solution of dihydro-beta-erythroidine hydrobromide[2] in physiological saline (1 cc/kg. body weight), the dose being sufficient to induce respiratory paralysis. Artificial respiration was then given by means of a respiratory pump.

Blood Gases and pH. The oxygen content and capacity and the carbon dioxide content of the blood were determined by the manometric methods of Van Slyke and Neill (11). Blood pH was determined anaerobically at $38°$ C. within a few minutes after the specimen was drawn, by means of a glass electrode and a voltmeter similar to that described by Nims (12). The standard buffers used were those of Hastings and Sendroy (13), since it was desired to calculate the carbon dioxide tension from the line charts of Van Slyke and Sendroy (14) based on the same pH scale. Root *et al.* (15) have shown that these charts are applicable to dog blood.

RESULTS

The cerebral arteriovenous difference in oxygen content was found to be greatly increased when the arterial carbon dioxide tension was decreased by hyperventilation. Likewise a decrease in the A-V difference occurred when the carbon dioxide tension was increased. In the 'normal' animals (breathing air), the A-V difference was found to be inversely related to the arterial carbon dioxide tension. In figure 1 are plotted all the available data except those in which the arterial oxygen tension was below 28 mm. Hg (calculated from the oxygen dissocation curve, with appropriate pH corrections). It is evident that when the oxygen supply is adequate the A-V difference in oxygen content is a function of the arterial carbon dioxide tension. The curve shows the upper and lower limits of the A-V difference, which may be interpreted as indicating the physiological limits of the cerebral vascular mechanism in response to changes in carbon dioxide tension.

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[2] Courtesy of Merck and Company, Inc., Rahway, N. J.

It is worthy of note that the paralyzing drug dihydro-beta-erythroidine hydrobromide is without noticeable effect on the responsiveness of the cerebral circulation to variations in carbon dioxide tension. In four animals of this group there was a tendency for the blood pressure to decrease to low levels during hyperventilation, but this was counteracted by the intermittent injection of adrenaline in amounts just sufficient to maintain the blood pressure in the normal range. In two hyperventilated animals the attempts to obtain sufficient blood from the sagittal sinus were unsuccessful, apparently as a result of extreme vasoconstriction and decreased blood flow.
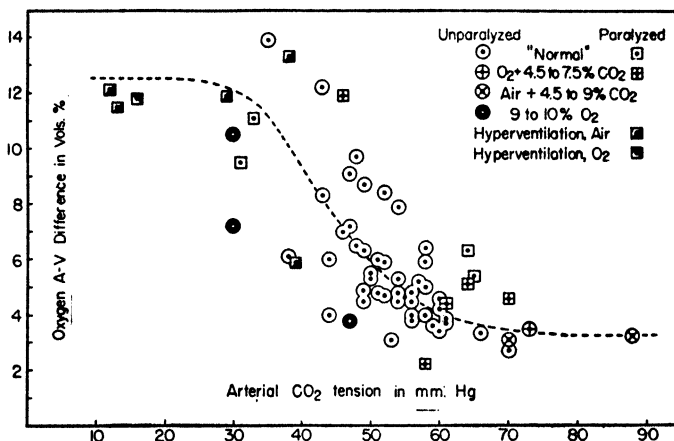
Fig. 1. RELATION OF THE CEREBRAL A-V OXYGEN DIFFERENCE to the arterial carbon dioxide tension.

DISCUSSION

Changes in the A-V oxygen difference induced by variations of carbon dioxide tension might be due to changes in either the cerebral blood flow or the cerebral oxidative rate, or to a combination of these factors. However, the available evidence indicates that changes in oxidative metabolism play only a minor rôle at most. Gibbs, Maxwell and Gibbs (3) found in the human being that increasing the carbon dioxide decreased the cerebral oxidations to some extent, but the effects of decreasing the carbon dioxide level were ambiguous. Schmidt (16) found that excess carbon dioxide decreased the apparent metabolism of the perfused dog's head in three of four experiments, but a similar effect induced by adrenaline was attributed to constriction of vessels in extracranial tissues. Kety and Schmidt (7) found in human subjects that active (voluntary) hyperventilation increased the cerebral oxygen uptake by 15 per cent, while passive hyperventilation caused no change. On the other hand, Geiger and Magnes (6), using the perfused cat's brain, found that increased carbon dioxide tension increased the oxygen consumption when the flow rate was originally low, the effect being secondary to an increase in blood flow. These findings appear to justify

the interpretation that the observed changes in the cerebral A-V oxygen difference resulting from alterations of carbon dioxide tension are due in large part to changes in blood flow through the cortex.

SUMMARY

Simultaneously drawn specimens of arterial and cerebral venous blood were obtained from morphinized dogs. Since the venous blood was obtained from the superior sagittal sinus it represents blood draining the cortex without admixture of significant amounts of extracranial blood. The specimens were analyzed and values calculated for carbon dioxide and oxygen tensions and for A-V difference in oxygen content. The effects on these variables of changes in the respiration or in the composition of the respired air were observed.

In the presence of adequate oxygen the cerebral A-V oxygen difference is a function of the arterial carbon dioxide tension. The A-V difference is high when the carbon dioxide tension is low, and vice versa. The data support the view of Lennox, Gibbs and co-workers that the brain is protected from rapid alteration of its acid-base balance by compensating changes in the cerebral blood flow.

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ACCELERATORY EFFECTS ON RENAL FUNCTION[1]

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PHYSIOLOGICAL stresses to which the organism is subjected in aviation are frequently referred to low barometric pressures or acceleratory forces which may be involved. Renal changes in animals exposed to simulated high altitudes have been investigated by one of us (1) and other work from this Laboratory has suggested that the kidneys may be affected during or after tests on acceleration. The present experiments were designed to investigate possible renal changes in function and structure resulting from exposure to various positive and negative acceleratory forces.

METHODS

Adult male white rats of Wistar strain were used throughout. Urine collection was made in small glass graduated cylinders under individual metabolism cages and the chloride content analyzed by the standard Volhard titration. Residual bladder urine was express⸴ ⸴ by manual pressure and massage technique.

The animals were subjected to acceleratory forces on a centrifuge of 10-feet radius (2). A special animal board which held 6 rats in the supine position was used; the strain on the body was partly borne by a soft leather band with two holes to accommodate the forefeet. Front and hind legs were extended and tied. In this position rats could be held without apparent discomfort for considerable periods of time if desired. During runs on the centrifuge the animals were covered by a cloth to protect against cooling by air draughts. Control tests showed that simply holding normal animals tied in the supine position for time periods similar to those of the experimental runs had no influence on fluid or chloride output. All injections were made intraperitoneally on the basis of body weight of the animal, with fluid (0.2% or 0.5% sodium chloride solution) warmed to body temperature.

The animal board was reversible and positive or negative g could be applied to the experimental group as a whole. A simultaneous paired control was run in each experimental group, all factors being duplicated except acceleration.

RESULTS

Positive g: urine secretion during acceleration. All animals were given 7.5 cc. of 0.5 per cent NaCl/100-gm. body weight in order to furnish ample fluid for secretion. At the end of two hours the urinary bladders were emptied by gentle pressure massage and the animals then tied down on boards in groups as indicated. One group was centrifuged at $2\ g$ for a period of 60 minutes, precaution being taken by suitable ligation (under novocaine) that urine was not voided during the exposure

TABLE I. ACCELERATION AND RENAL SECRETION. POSITIVE (+) G FORCES

Group I[1]

NO. TESTS	EXPERIMENTAL CONDITION	URINE OUTPUT	
		cc. in 60 min.	% of injected fluid
10	Control (no accel.)	1.1	18.2
11	Acceleration	0.2	1.7

Group II[2]

NO. TESTS	EXPERIMENTAL CONDITION	URINE EXCRETION AT END OF		5-HR. CHLORIDE EXCRETION
		3 hrs.	5 hrs.	
		cc.	cc.	mg.
6	Control (no accel.)	0.2	0.6	3.1
5	Acceleration	0.6	0.9	3.8

Group III[3]

SERIES	NO. TESTS	EXPERIMENTAL CONDITION	URINE EXCRETION AT END OF				
			2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
			cc/100 gm. b.wt.	cc/100 gm. b.wt.	cc/100 gm. b.wt.	cc/100 gm. b.wt.	cc/100 gm. b.wt.
A	12	Control (no accel.)	1.6	1.9	2.6	3.0	3.5
	12	Acceleration (repeated)	1.9	2.4	2.9	3.1	3.5
B	21	Control (no accel.)	1.0	1.6	2.1	2.8	3.5
	22	Acceleration (contin.)	1.4	2.3	3.0	3.6	4.3

Group IV[4]

SERIES	NO. TESTS	EXPERIMENTAL CONDITION	3-HOUR URINE OUTPUT	CHLORIDES	
				Concentration	Total 3-hr. excretion
			cc/100 gm. b.wt.	mg/cc.	mg.
A	12	Control (no accel.)	2.3	0.59	1.4
	12	Acceleration (repeated)	3.0	0.55	1.7
B	28	Control (no accel.)	2.1	1.16	2.5
	28	Acceleration (contin.)	2.8	0.89	2.5

[1] Animals given 7.5 cc. 0.5% NaCl/100 gm. b. wt.; 2 hr. later, experimental rats exposed to 2 g for 60 min.

[2] *Ad lib.* fluid intake; exposure, 5 g for 15 sec., 30 times at 1-min. intervals.

[3] Accel. (*A*), 5 g for 15 sec., 15 runs at 1 min. intervals, or (*B*) 2 g for 15 min.; rats injected with 5 cc. 0.5% NaCl/100 gm. b. wt.

[4] Accel., 5 g for 15 sec., (*A*) repeated 25 times at 1 min. intervals, or (*B*) 2 g for 15 min.; rats given 5 cc. 0.2% NaCl/100-gm. b. wt.

period; the others were similarly tied down only to serve as non-accelerated controls. At the end of the 60-minute experimental period the rats were killed, the urinary bladders clamped off and removed and the contained fluid measured. From the

results shown in table 1 *I* and figure 1, it will be observed that during exposure to *g* forces urinary secretion is virtually stopped.

Urine secretion after exposure to g.   Rats which had been allowed free access to food and water were *a*) centrifuged for 15 minutes at 2 *g*, or *b*) given 30 runs at 5 *g* for 15 seconds at 1-minute intervals; each group was then placed in metabolism cages. There was no significant difference between the urine output of *group a* rats and their controls, but in the *b* series there was a greater post-acceleratory renal output in the experimental animals at the end of three hours.   During the succeeding two hours, accelerated and control animals both excreted about the same amount of urine.   No
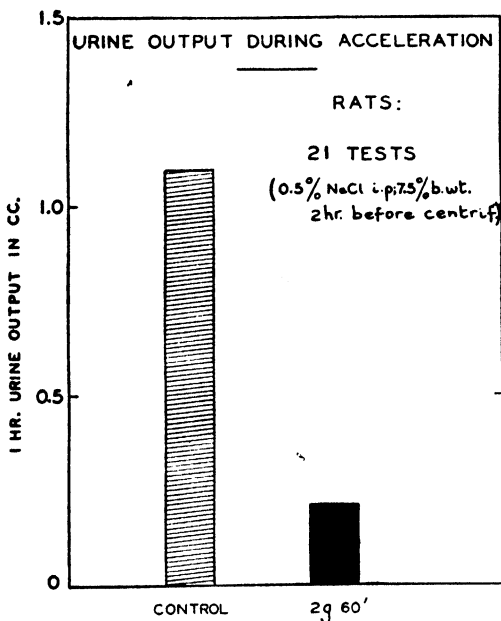
Fig. 1

significant difference was noted, also, between the five-hour chloride output of the two groups (table 1, *II*).

Though the percentage increase in urine output of accelerated animals (5 *g*, 15 sec., 30 runs) over the controls was high, the actual quantity of urine excreted was of a small order on an *ad libitum* water intake.   A group of animals was therefore rendered diuretic by the injection of sodium chloride solutions immediately after exposure to *g* forces and before placing them in metabolism cages.

Groups of animals were exposed 15 times to 5 *g* for 15 seconds at one-minute intervals, then injected intraperitoneally with 5 cc. of 0.5 per cent NaCl/100 gm. body weight and placed in metabolism cages.   Non-accelerated controls were similarly injected and the urine output was followed during the succeeding six hours. Other animals were subjected to 15 minutes' acceleration at 2 *g* (fig. 2).   Results are given in table 1, *III, A* and *B*,   Urinary secretion of the repeatedly accelerated animals was considerably higher than that of the controls over several hours in the *A*

series, while in the *B group* post-acceleratory output was maintained at about 40 per cent higher over the six-hour observation period.
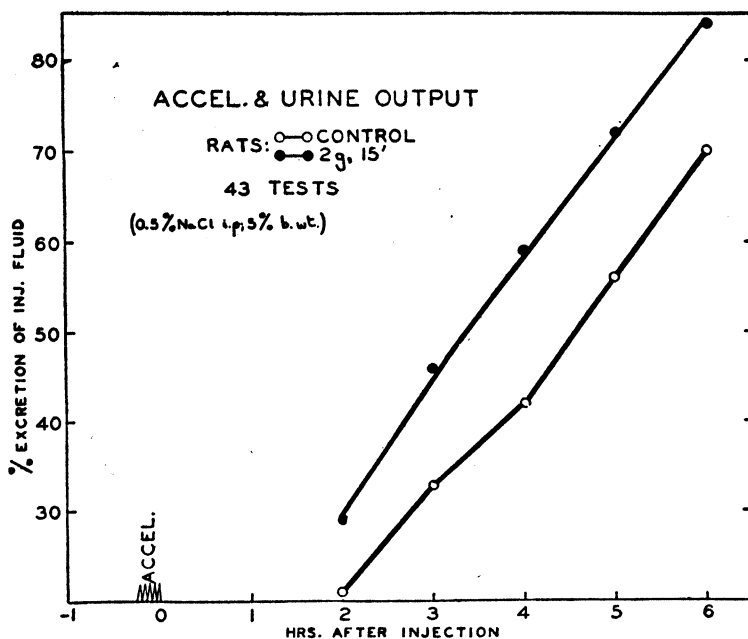
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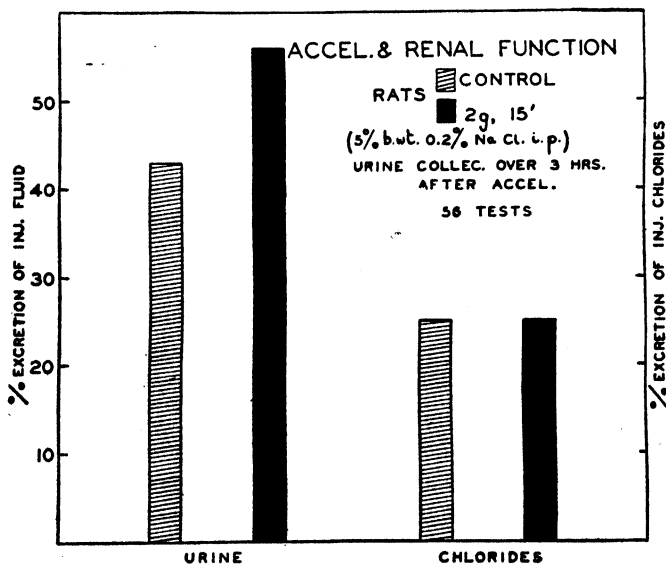
Fig. 3

Animals subjected to either repeated acceleration (5 g, 15 sec. for 25 runs at 1-min. intervals) or a continuous exposure period (2 g for 15 min.) were injected with

5 cc. of 0.2 per cent NaCl/100 gm. body weight, placed immediately in metabolism cages and the urine collected and analyzed for chlorides at the end of three hours. Urine output was increased over the similarly injected controls and chloride concentration diminished in both series (table 1, *IV* and fig. 3).

Effect of long periods of acceleratory exposure. Several groups of animals were exposed to various positive and negative g forces over periods of 8 to 24 days (daily

TABLE 2.   RENAL AND OTHER CHANGES FOLLOWING ACCELERATORY EXPOSURE

| SERIES	NO. RATS	EXPERIMENTAL CONDITION	B.WT. AT BEGINNING OF EXPOSURE	B.WT. AT END OF EXPOSURE	GAIN IN B.WT.	WT. BOTH KIDNEYS	KIDNEY WT/B.WT.
			gm.	*gm.*	%	*gm.*	
A	5	Control (no accel.)	190	249	31	2.02	0.81
	6	Acceleration (+2 g for 15 min.; 17 days)	178	205	16	1.72	0.84
B	6	Control (no accel.)	192	212	10.4	1.63	0.77
	6	Acceleration (−4 g for 15 min.; 17 days)	187	196	4.8	1.49	0.76
C	3	Acceleration (+6 g, 15 sec. 3 runs daily, 8 days)	229	237	3.5	1.70	0.72
	3	Acceleration (+6 g, 15 sec. 3 runs daily, 24 days)	227	254	11.8	1.93	0.76

TABLE 3.   ACCELERATION AND KIDNEY FUNCTION: EFFECT OF PITRESSIN[1]

SERIES	PITRESSIN	CONTROLS (NO ACCELERATION)		ACCELERATION (2 G, 15 MIN.)	
		3-hour urine output	Urinary chlorides	3-hour urine output	Urinary chlorides
	meq.	*cc/100 gm. b.w.*	*mg/cc.*	*cc/100 gm. b.w.*	*mg/cc.*
1	0	2.1	0.33	2.7	0.48
2	4	1.3	0.71	2.1	0.63
3	8	1.1	2.54	1.8	1.52
4	16	0.4	2.62	1.9	1.64
5	32	0		0.8	2.40
6	63	0.4	8.80	0.4	7.11
7	125	0.6	11.6	0.7	10.6
8	250	1.4	8.31	1.5	9.12
9	500	1.8	8.67	1.7	8.00
10	1000			1.3	9.32

[1] All animals given 5 cc. 0.2% NaCl/100-gm. b. wt. plus amount of pitressin noted; 6 or more tests in each series.

tests, except week-ends). At the end of these periods the animals were killed and the kidneys removed, weighed and sectioned for histological analysis. During the experimental period, the exposed animals gained less weight than the controls, although the ratio of kidney to body weight was insignificantly higher (table 2, *A*, *B*, *C*). Tests for urinary albumen at the end of the test periods were almost invariably positive in these cases. Histological examination revealed that the renal glomeruli

of the accelerated animals were ischemic and the proximal convoluted tubules showed cloudy swelling and contained casts. The loop of Henle was apparently unaffected.

Effect of post-pituitary extract on acceleratory polyuria. Groups of animals were subjected to acceleratory forces of 2 g for 15 minutes, injected with 5 cc. of 0.2 per cent NaCl solution, containing various amounts of pitressin, and then placed in metabolism cages for a period of three hours. At the end of this period the urine was measured and its chloride concentration determined (table 3).
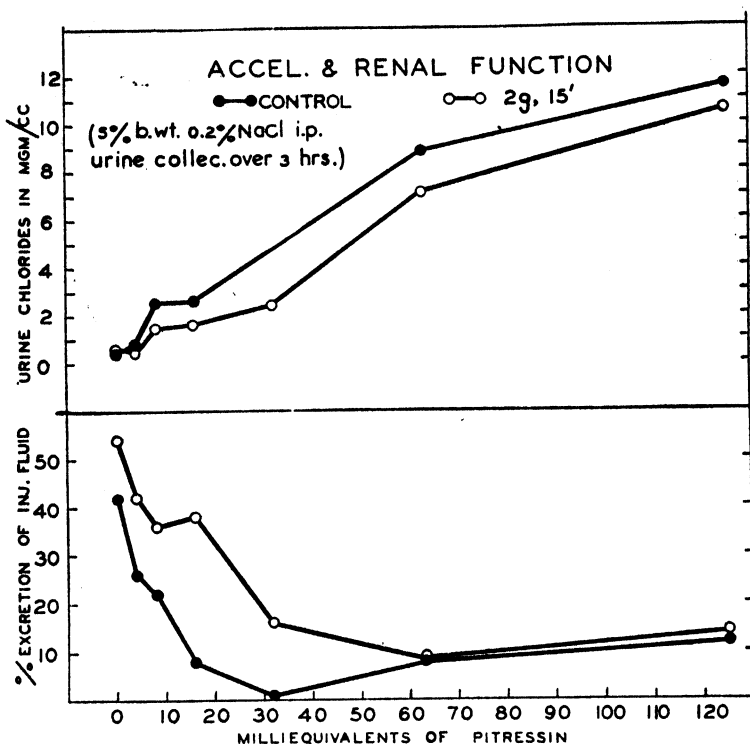
Fig. 4

Curves showing the effect of post-pituitary extract on the urine and chloride output of non-accelerated rats followed the usual pattern previously observed and described by one of us (3). With decreasing doses of pitressin, the urine output gradually rose while its chloride concentration fell. The most abrupt fall in chloride concentration was observed in earlier work on using a pitressin concentration of approximately 4 mU/100-gm. rat. In the present tests a similar sharp fall in chloride concentration of the urine taken from accelerated rats paralleled closely (though it was somewhat below) that of control animals (fig. 4).

As the dose of pitressin was decreased (for convenience the dose was halved in each successive experiment), the curves of urine secretion of accelerated and control animals began to diverge on using approximately 60 mU. (fig. 4). At this point acceleratory polyuria 'broke through' post-pituitary inhibition of urine flow. It may

be noted that with this concentration of pitressin, urinary chlorides were still unaffected. Thus in both non-accelerated and accelerated animals the chloride-concentrating power of the hormone was better maintained than its water-concentrating power.

Negative g: urine secretion during acceleration. In these experiments the factors of technique time, fluid injection and metabolic period were the same as those previously described under positive g, but the animals were exposed to −4 g instead of +2 g for 60 minutes. The results were similar, i.e. during exposure to negative g (as well as positive g) urine secretion was almost completely suppressed (table 4, *I*).

TABLE 4. ACCELERATION AND RENAL SECRETION. NEGATIVE (−) G FORCES

Group I[1]

NO. TESTS	EXPERIMENTAL CONDITION	URINE OUTPUT	
		cc.in 60 min.	% of injected fluid
4	Control (no accel.)	1.5	10.0
6	Acceleration	0.2	1.4

Group II[2]

NO. TESTS	EXPERIMENTAL CONDITION	3-HOUR URINE OUTPUT	CHLORIDES	
			Concentra'.n	Total 3-hr. excretion
		cc/100 gm. b.wt.	mg/cc.	mg.
18	Control (no accel.)	2.2	1.39	3.1
18	Acceleration	3.1	0.52	1.6

[1] Animals given 7.5 cc. 0.5% NaCl/100-gm. b. wt.; 2 hours later, experimental rats exposed to −4 g for 60 min.

[2] Accel., −4 g, 15 min.; animals given 5 cc. 0.2% NaCl/100-gm. b. wt. i.p.

Urine secretion following negative acceleration. Rats which were exposed to −4 g for 15 minutes, then injected with 5 cc. of 0.2 per cent NaCl/100-gm. body weight and placed in metabolism cages for three hours, excreted more urine and less chloride than their controls (table 4, *II*). The increase in polyuria over the controls was 36 per cent after exposure to negative g, 33 per cent after positive g—an insignificant difference. Urinary chloride concentration was reduced 62 per cent from the control level following −4 g, only 23 per cent after −2 g—a difference which appears to be significant.

DISCUSSION

Although the acceleratory exposures given in the present experiments were usually more severe than those experienced by man, they could not be considered critical for rats. No animal died from the acceleration and the general condition of animals following the tests was excellent, even when exposures were made daily over periods of a few weeks. Considered in relation to earlier results on the effects of

high altitude exposure on kidney function and renal pathology, the results of acceleratory exposures appear very interesting and significant.

Rats exposed to low barometric pressure (25,000 ft. equiv. alt.) have been observed to suffer much greater polyuria than those subjected to high acceleratory forces; also, the pathological changes in the kidneys following repeated exposure to each condition were strikingly different. The kidneys of 'high-altitude rats' were greatly hypertrophied, the glomeruli were congested and slight tubular pathology was observed. In the case of the g-exposed rats, however, there was little or no gross renal hypertrophy, the glomeruli were characterized by almost complete absence of blood and the tubules by cloudy swelling, while the loop of Henle presented a normal appearance.

It appears difficult to explain or correlate the pathological changes observed after repeated exposure to acceleration with the changes in renal function as reported herein. However, the functional changes described would seem to be referable to both glomerular and tubular impairment consequent upon disturbances in renal circulation produced by high acceleratory forces, with resultant anoxia of the intimate renal tissues.

Changes in kidney secretion observed following centrifugation, it should be considered, may be due either to a) stimulation and enhanced secretion of the corticoadrenal tissues or b) to central inhibition of post-pituitary function. The former factor a) is probably important in all cases of stress and may bring about a significant diuresis, while b) diminished secretion of the antidiuretic, chloruretic hormone would also tend to produce the copious, dilute urine observed in our experiments. The acceleratory polyuria is readily inhibited or reversed by the injection of adequate doses of post-pituitary extract. This, however, does not necessarily implicate the pituitary gland in the reno-functional changes observed.

SUMMARY

The effects of acceleratory forces on renal function and pathology have been studied in the rat. The various degrees of exposure used were not evidently disturbing to the animal, although rather severe by human standards. During exposure to either positive or negative g forces, urinary secretion was suppressed. Following centrifugation the volume of urine excreted was increased above normal up to 40 per cent for five to six hours and its chloride concentration was reduced in comparison with non-accelerated controls. Acceleratory polyuria was reversed or inhibited by posterior pituitary extract. After repeated daily exposure to acceleratory forces over a period of about three weeks, albumin was present in the urine and significant pathological changes were found in the rat kidney. The effects are probably referable to anoxia brought about by reno-circulatory disturbances under high acceleratory forces. Cortico-adrenal and post-pituitary functions may be involved.

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ELECTROPHRENIC RESPIRATION. III. MECHANISM OF THE INHIBITION OF SPONTANEOUS RESPIRATION[1]

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I N PREVIOUS communications it was demonstrated that artificial respiration could be produced by electrical stimulation of one or both phrenic nerves, with a series of impulses the voltage of which rose and fell in such a way as to produce effective diaphragmatic contraction and relaxation (1–3). It was found that smooth respiratory activity could be so induced and that the technique was capable of maintaining normal oxygen and carbon dioxide partial pressures in the arterial blood of the experimental animal and man in the absence of spontaneous respiration. The reserve of the technique was sufficient to permit hyperventilation and the production of alkalosis with stimulation of only one phrenic nerve.

An interesting observation made during the original study was that spontaneous respiration ceased within seconds after the onset of electrical artificial respiration. This observation was deemed to be of potential clinical importance, because of the manner in which patients not infrequently interfere with other types of artificial respiration. This is particularly true in patients with bulbar poliomyelitis. This report presents an examination of the mechanism by which spontaneous respiration is inhibited when electrical artificial respiration is started in the experimental animal.

METHODS

Dogs of both sexes, weighing from 11 to 14 kg. were studied. Nembutal anesthesia, 30 mg. to 40 mg/kg. of body weight, was used. Electrophrenic respiration was applied by means of the apparatus previously described (2). Respiratory movements were recorded by means of a corrugated rubber tube placed around the lower part of the rib cage. Changes of pressure in the tube were registered on a direct-writing galvanometer by means of the electromanometer[2] in use in this laboratory. These pneumograms registered both diaphragmatic and intercostal activity but did not do so in a quantitative manner.

The partial pressures of oxygen and carbon dioxide in arterial blood were determined according to the technique of Riley (4). All determinations were done in duplicate immediately after the blood sample was drawn. Minute volumes were obtained with a spirometer. Vagus section was performed low in the neck.

The experiments described below were designed to yield data which might help answer five main questions: 1. Is all spontaneous respiration inhibited immediately after the onset of electrophrenic respiration? 2. If electrophrenic respiration does

inhibit spontaneous respiration, is the suppression of chemical or neurogenic origin? 3. If the inhibition is of a reflex nature, what is the afferent pathway over which the reflex travels? 4. After interruption of the afferent pathway, can spontaneous respiration still be inhibited by overventilation? 5. Is the reflex inhibition of spontaneous respiration vigorous enough to inhibit spontaneous respiration in the presence of a strong chemical respiratory stimulus?
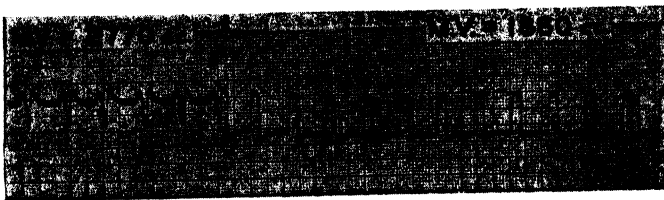

Fig. 1. PNEUMOGRAM OF ONSET OF ELECTROPHRENIC RESPIRATION. Artificial respiration started at signal. M.V. = min. vol. before and during electrophrenic respiration. Larger deflection during electrophrenic respiration does not indicate deeper respiration but does indicate stronger diaphragmatic contraction. Downward deflection registers inspiration and the same is true for subsequent figures. Paper speed is 2.4 mm/sec.
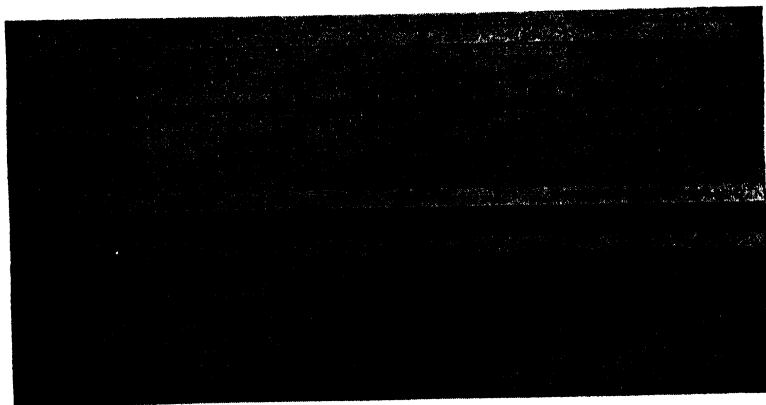

Fig. 2. SUSTAINED DIAPHRAGMATIC CONTRACTION: *A*, vagi intact; *B*, vagi cut. Sudden continuous and sustained contraction of diaphragm was started at the first signal and discontinued at the second in both tracings. In tracing *A*, before the vagi were cut, all effective intercostal respiration was inhibited by keeping the diaphragm contracted. In tracing *B*, after vagotomy, the intercostal rhythm was not suppressed by the same degree of sustained diaphragmatic contraction.

RESULTS

1. *Immediate inhibition of spontaneous respiration.* Figure 1 is an example of the immediate cessation of spontaneous respiratory effort which occurred in a dog immediately after the onset of electrophrenic respiration. The pneumogram pattern shows a machine-like regularity during electrophrenic respiration and contains no evidence of interference by spontaneous respiratory efforts. This cessation of spontaneous respiration occurred despite the fact that the respiratory rate was unchanged and the minute volume was lower than that observed before the onset of electrophrenic respiration. That spontaneous breathing is really inhibited, and not

just masked by electrophrenic respiration, is borne out by the fact that, when normal or greater than normal minute volumes are maintained for only a fraction of a minute by the electrophrenic respirator, a brief period of apnea follows the cessation of stimulation. A similar observation was made in man (3).

2. *Neurogenic inhibition of breathing by sustained diaphragmatic contraction.* In figure 2A can be seen the effect of continuous electrically sustained diaphragmatic contraction. No spontaneous respiratory effort was made until just before the stimulation stopped. During a period of similar diaphragmatic contraction performed a short while before the taking of the tracing in figure 2A, the arterial partial pressure of CO_2 changed from 37 mm. Hg before stimulation to 41 mm. Hg 50 seconds after the application of the stimulus. Oxygen partial pressure changed from 84 to 60
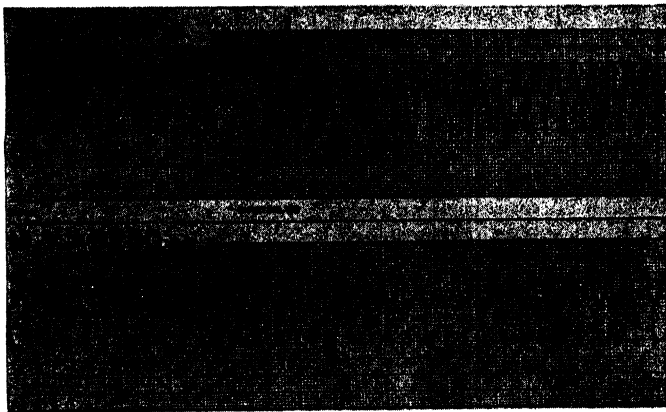

Fig. 3. OVERVENTILATION BY ELECTROPHRENIC RESPIRATION after vagi have been cut. Electrophrenic respiration started at the signal. M.V. = min. vol. before and after the onset of electrophrenic respiration. Tracings are continuous. No further irregularities of the respiratory pattern occurred after the one seen in the middle of the lower tracing.

mm. Hg. Despite this rise in CO_2 and fall in O_2, no spontaneous respiratory effort was made during this 50-second period.

3. *Afferent pathway of the inhibitory reflex.* The tracing in figure 2B, taken 12 minutes later, shows the result of the same procedure 7 minutes after section of both vagus nerves. It can be seen that spontaneous respiratory movements continued at the same rate throughout the period of stimulation. They are, however, of smaller amplitude than before stimulation, since the electrical splinting of the diaphragm confines respiratory activity to the intercostal muscles during the stimulus period.

4. *Effect of overventilation after vagotomy.* In another experiment, with sectioned vagi, after setting the electrophrenic respirator for an appreciably higher respiratory rate and minute volume than the dog's spontaneous rate and minute volume, electrophrenic respiration was started. Figure 3 shows the result. It can be seen that spontaneous respiration was not immediately inhibited and subsided only after a period of overventilation had occurred.

5. *Reflex inhibition of respiration during inhalation of CO_2.* A dog was made to breathe 7 per cent carbon dioxide in 93 per cent oxygen. The electrophrenic respi-

rator was set for a rapid deep respiration and started 12 minutes after the dog had begun breathing the above gas mixture. Figure 4 shows the result. It can be seen that the respirator assumed immediate control and inhibited spontaneous respiration while a high level of carbon dioxide (71 mm. Hg) was present in the arterial blood. The presence of barbiturate anesthesia may have decreased the sensitivity of the respiratory center to carbon dioxide. It cannot be doubted, however, that reflex suppression of spontaneous respiration was an important element, for, while still breathing 7 per cent CO_2, after cutting the vagi, definite interference with the respirator's control occurred, as is evident from figure 5. This interference with the respirator's control persisted for the duration of the observation period (15 min.).

Fig. 4. ELECTROPHRENIC RESPIRATION during administration of 7% CO_2. The above tracing was taken 12 min. after the dog was made to breathe 7% CO_2 in 93% O_2. During this period the arterial blood CO_2 partial pressure was 71 mm. Hg. Rapid, deep, electrophrenic respiration was begun at the signal and assumed control of respiration.
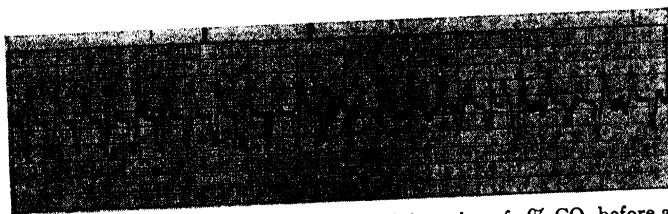

Fig. 5. ELECTROPHRENIC RESPIRATION during administration of 7% CO_2 before and after vagus section. The same preparation as in fig. 4. Dog still breathing 7% CO_2 in 93% O_2 and is still under the control of the electrophrenic respirator at the beginning of the record. Left vagus cut at the first signal and right vagus cut at the second signal. Interference with the electrophrenic control of respiration by spontaneous respiratory motions is apparent immediately after vagotomy.

This experiment also confirmed the fact that the initial immediate suppression of respiration in the previous experiments (fig. 1) was not due to the excessive elimination of carbon dioxide.

<div align="center">DISCUSSION</div>

The data presented demonstrate that spontaneous respiration ceases immediately after the onset of electrophrenic respiration. In previous experiments on the dog, cat, rabbit and monkey, this phenomenon was also observed. That this inhibition has two components has been demonstrated above. The first of these is a reflex set up by diaphragmatic contraction. At least a portion of the reflex has its afferent pathway in the vagus nerves. For with intact vagi, a sustained diaphragmatic contraction reflexly inhibits respiration even though the arterial oxygen falls and carbon

dioxide rises (fig. 2A). After section of the vagi this phenomenon no longer occurs (fig. 2B).

The second component in the inhibition of spontaneous respiration is the change in blood chemistry brought about by electrophrenic respiration, if the respiration thus induced is sufficient to overventilate the animal. That this component can also be effective in inhibiting respiration in the absence of the reflex component is demonstrated by figure 3.

The potency of the reflex inhibition of spontaneous respiration by electrophrenic respiration can be deduced from those experiments in which, despite an extreme level of carbon dioxide present in the blood, spontaneous respiration did not occur while vigorous electrophrenic respiration was in effect (fig. 4).

We have been vague concerning the precise location of the sense organs initiating the impulses that inhibit spontaneous respiration when electrophrenic respiration is applied. It is, of course, likely that the Hering-Breuer reflex, as it is customarily interpreted (5), plays a part in the reflex inhibition of respiration as described above. However, certain proprioceptive sense organs other than those which send impulses up the vagus nerve may prove significant and to ascribe the effect solely to stretching of the pulmonary parenchyma may be to overlook certain other significant contributing afferents. The fact that forceful endotracheal insufflation does not cause a comparable immediate reflex inhibition of respiration indicates that simple stretch of the pulmonary parenchyma is not the only factor involved in the reflex inhibition described in the above experiments. Simple stretch of the pulmonary ligament has, under certain conditions, been found to inhibit inspiration and to initiate a forceful sustained expiratory effort. This matter is under investigation at the present time.

SUMMARY

The inhibition of spontaneous respiration by electrophrenic respiration has two components. The first is neurogenic and the second chemical. The first, reflex in nature, consists, in part, of impulses that travel up the vagus nerve during the period of diaphragmatic contraction. This reflex disappears after sectioning the vagi. The reflex is immediate if the initial diaphragmatic contraction is adequate. In addition to the reflex inhibition, spontaneous respiration can be inhibited by providing electrophrenically induced overventilation. The potency of the neurogenic inhibition has been demonstrated by the fact that it can inhibit spontaneous respiration in the presence of a vigorous chemical stimulus to respiration, namely a high carbon dioxide partial pressure in the arterial blood.

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FLUID SHIFTS IN ANIMALS DURING PRESSURE BREATHING[1]

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THE use of high positive pressure respiration, combined with adequate counter-pressurization presents a novel environment for man and animals. Much of the physiology of pressure breathing has recently been reviewed by Barach *et al.* (1). Work in our laboratory has been directed towards the specific problems of fluid dynamics during exposure to high positive pressure respiration. The first of these studies by Henry *et al.* (2) is concerned with the measurement of fluid loss in men under these conditions. We have made a parallel study of the circulatory effects of high pressure breathing with partial protection in cats. Interest was focused on the rate and amount of fluid loss as indicated by hemoconcentration, the extent of protein leakage from the circulation and the kinetics of disappearance of an injected dye.

The methods developed for this study are applicable to use in quantitative investigations of vascular filtration. Unlike the venous occlusion technique, the pressures attainable are theoretically unlimited, since pressure breathing theoretically increases both the venous and the arterial pressures. It is therefore possible to achieve any reasonable pressure level in the capillaries of the exposed limbs and thus provide extremely high filtration pressures. Since all four limbs (or any number of limbs) may be left unprotected, the area for filtration is considerably greater than in the case of occlusion of a single extremity and therefore significant fractions of the total circulating volume may be driven from the circulation.

METHODS

Cats weighing between 2.4 and 4.7 kg. were used as experimental animals. They were intravenously anesthetized with pentobarbital (ca. 35 gm/kg. body wt.) and were given an equal maintenance dose subcutaneously. The animals were then prepared by exposure of the large blood vessels in the femoral region and the insertion of sub-dermal electrocardiogram leads.

Protection was provided by counter-pressurization of the thoracic and abdominal areas with an air bladder and non-distensible vest. The bladder was made of two thicknesses of vinylized nylon cloth, with appropriate openings for all four limbs, and an opening along the mid-dorsal line. (See insert, fig. 1). The stitched edges were made air-tight by sealing with rubber cement. Pressurizing gas was conducted into the bladder through an aluminum bushing, bolted and cemented into the external layer only. The jacket design closely followed the same pattern, except that it was made of a single layer of 'Berger' cloth and was provided with a tongue and

eyelets along the dorsal opening to provide an adjustable closure by lacing.   In addition, suitable straps and buckles were sewed to the anterior end to give a better fit around the neck and as points of attachment for the helmet.   This system provides protection for all of the body with the exception of the head and the four limbs.

In the earlier studies tracheal cannulation was used and partial protection for the head was achieved by tight binding with elastic bandages.   This technique was not entirely satisfactory, since it required surgical manipulations in the neck and there was no assurance that counter-pressurization of the head had been adequate.   The system was therefore modified to include a lucite helmet, which was large enough to encase the entire head and which was sealed by means of a vinylized nylon 'skirt' which could be slipped between the air-bladder and the vest at the neck (cf. fig. 1).
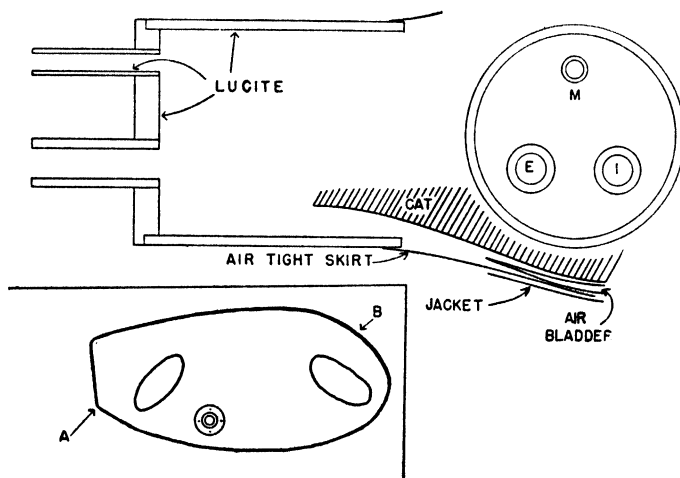

Fig. 1. DETAILS OF PRESSURE BREATHING HELMET and counter-pressurization system.   Method for making air tight joint between helmet and cat is shown in lower right.   *Insert:* pattern, much reduced, for the counter-pressurization system. E, connection to expiratory system; I, connection to inspiratory system; M, connection to manometer.

This system provided satisfactory counter-pressurization for the head and neck and in extreme trials showed no important leaks at pressures as high as 180 mm. Hg.

The pressure breathing system consisted of two diaphragm-type pressure regulating valves, connected on their high pressure side to a source of compressed air or oxygen.   The output of one regulator (R_i of fig. 2) was connected directly with a solenoid valve (V_i); the output of the other regulator (R_e) was connected to the loading port of a modified Linde mask exhalation valve (L).   The exhalation port of the Linde valve was connected to a second solenoid valve (V_e).   These electrically operated valves were then connected either to the tracheal cannula or to the pressure helmet.   The protective counter-pressurization bladder was connected to the respiratory circuit at some point between the two solenoid valves.   The timing and alternation of inspiration and expiration was achieved by alternately opening the two solenoid valves.   A suitable telechron driven cam (S) operated a switching system to give a respiratory rate of 60 (or in later experiments 30) cycles/min., with approximately two-thirds of each cycle in inspiration and one-third in expiration.

The arrangement of the several pressure and electrical circuits are schematically represented in figure 2.

General Procedure. Immediately after completing the preparation, a single control sample was taken from the femoral artery. Soon thereafter about 0.25 mg. of the dye T-1824/kg. body weight was injected into the femoral vein. At precisely measured intervals of about 15 to 30 minutes after the dye injection, further arterial blood samples were taken. Thirty to 70 minutes later the animal was disconnected from the pressure system and samples were taken during the subsequent 90 minutes.
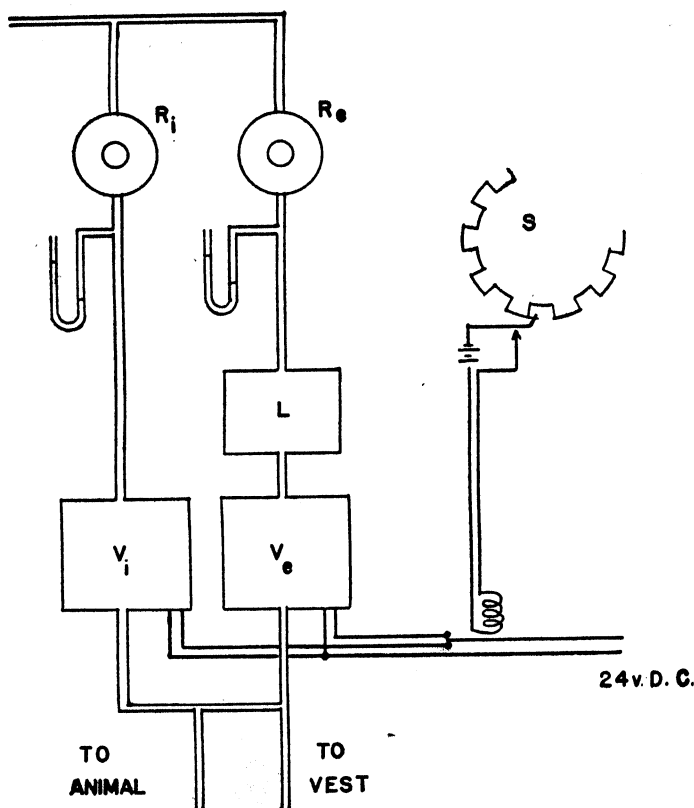
Fig. 2. SCHEMATIC REPRESENTATION of the pressure breathing system.

Analytical Techniques. Fluid shifts were determined from four sets of data: hematocrit, hemoglobin, plasma protein concentration and dilution of the injected dye. For each sample, one ml. of blood was taken into a syringe containing 1.6 per cent sodium oxalate. The amounts of oxalate and of blood were determined gravimetrically and were approximately in a ratio of 1:5. After thorough mixing, ca. 0.8 cc. was delivered into a Wintrobe hematocrit tube. The remainder, 0.3 to 0.4 cc., was utilized for hemoglobin determinations by the colorimetric acid hematin technique. The hematocrit values were determined directly by the method of Wintrobe (3). The supernatant plasma in the hematocrit tubes was removed by pipette. Exactly 0.2 ml. of this plasma was diluted with 1.0 ml. of 0.9 per cent

saline. The dye concentrate in this diluted plasma was then determined with a Beckman quartz spectrophotometer. Plasma protein concentrations were determined by the falling drop method of Barbour and Hamilton (4) on another aliquot of the supernatant fluid in the hematocrit tube. All data were corrected for dilution of oxalate and saline.

<div align="center">RESULTS AND DISCUSSION</div>

A total of 48 cats were used in this study. Of these, 7 animals were exposed to pressure breathing in the helmet described, while 37 experiments were performed with tracheal cannulation and the remaining animals served as controls for the sampling techniques. Of the 44 animals exposed to pressure breathing, 30 survived the entire experiment, including a post-experimental period of 30 to 60 minutes. The fatalities could usually be attributed to accidents or to some obvious fault in the technique. Since adequate data were not obtained in all cases, the number of animals on which results are cited will be less than these totals.

No significant differences were noted between the data obtained with the pressure helmet and those obtained in the experiments with tracheal cannulation, therefore all of the results are treated together in table 1. The percentage of fluid loss was calculated from the average prepressure breathing values and the highest values of hematocrit or hemoglobin obtained during the stress. The calculations were made on the assumption of a constant volume of circulating erythrocytes throughout the experiment. Plasma protein leakage was judged qualitatively from the relationship between the hematocrit shift and the plasma protein concentration shift during the same period. The ratio of initial hematocrit to initial plasma protein level is presented as a rough index of the condition of the animal at the beginning of the experiment. While a low hematocrit might result from over-hydration, a low hematocrit:plasma protein ratio would suggest a real anemia. The respiratory pressure given is the arithmetic mean of the inspiratory and expiratory pressures used. This value probably is somewhat smaller than the true value, since the duration of the inspiratory (high pressure) was in all cases greater than the expiratory phase. The inspiratory pressures ranged from 22 to 120 mm. Hg, while expiratory pressures were between 40 and 60 mm. Hg.

In spite of the wide variation in the individual values, it will be noted that there is a tendency towards greater fluid loss in animals subjected to the higher pressures (*Group B* as compared with *Group A*). The animals in *Group C* are all characterized by a somewhat lower hematocrit:protein ratio, and showed abnormally high fluid loss, with a consistent tendency for protein leakage.

The kinetics of the fluid shifts may be judged from figures 3 and 4. In these figures the hematocrit, hemoglobin and plasma protein values are plotted as percentage of the control values at the times of the several samples. Figure 3, representing data on a cat with a high hematocrit:protein control ratio, shows a moderate fluid loss and negligible protein leakage. The data in figure 4 were obtained on a cat with a low ratio (representative of *Group C*) and show a marked fluid loss with a strong tendency for protein leakage. The curves representing the disappearance of dye from the circulation showed no important deviations from the control when properly corrected for the fluid shifts.

In general, our results indicate 1) a fluid loss, the magnitude of which is, at least in part, dependent on the mean pressure employed in the respiratory system;

TABLE 1. FLUID LOSS FROM THE CIRCULATION OF CATS DURING PRESSURE BREATHING

ANIMAL NO.	WEIGHT	FLUID LOSS[1]		PROTEIN[2] LEAKAGE	MEAN RESP.[3] PRESSURE	HCRT/PROTEIN[4]
		HCRT	Hb			
	kg.	*per 100 cc.*			*mm. Hg*	
		Group A				
31	2.5	3.8	5.5	±	33	70.8
34	2.5	5.9	6.4	+	35	78.4
35	3.4	1.0	2.7	?	37.5	
36	3.3	8.3	8.6	−	37.5	62.7
Av........		4.75	5.8		35.7	70.9
		Group B				
6	2.9	11.3	13.6	?	48	
7	3.1	16.3		?	48	
8	2.4		15.4	?	47	
11	3.1	11.8	14.2	+	48	58.0
13	2.9	7.8	12.4	?	43	
14	2.9	12.9	13.3	−	43	65.7
16	2.8	11.3	12.2	−	42	62.4
21	?	12.7		±	42	63.4
24	2.4	9.4	9.6	?	42	
41	2.3	10.3		±	47.5	84.6
42	2.3	10.5		−	47.5	77.6
43	2.3	9.2	8.5	−	41.5	55.3
45	3.4	3.5	4.4	−	45	55.7
Av........		10.6	8.8		45	65.4
		Group C				
15	3.5	22.5	20.7	+	43	53.9
17	3.5	30.5	31.2	+	43	52.0
20	?	17.9	16.1	±	42	56.1
32	2.8	32.7	28.9	+	35	46.8
37	4.7	28.3	32.7	+	37.5	39.4
Av........		26.4	25.9		40.1	49.7

[1] Fluid loss was calculated from the av. pre-experimental hematocrit or hemoglobin values and the highest value observed during pressure breathing. The peak hematocrit and hemoglobin values were usually obtained in the same blood sample. More than 3/4 of the fluid was lost in 20 mins. after beginning pressure breathing.
[2] Protein leakage was judged as described in the text. A + indicates marked leakage; ± intermediate; and − indicates no leakage.
[3] The arithmetic mean of the inspiratory and expiratory pressures imposed.
[4] Ratio of the control hematocrit and plasma protein levels.

2) no consistent increase in protein leakage as a consequence of the pressure breathing per se; and 3) no fundamental change in the dye-disappearance curves. These re-

sults are concordant with the theoretical predictions based on increased capillary hydrostatic pressure. They suggest that the simple distention of the smaller blood vessels evoked by the procedures used are not of sufficient magnitude to alter their normal impermeability to proteins.
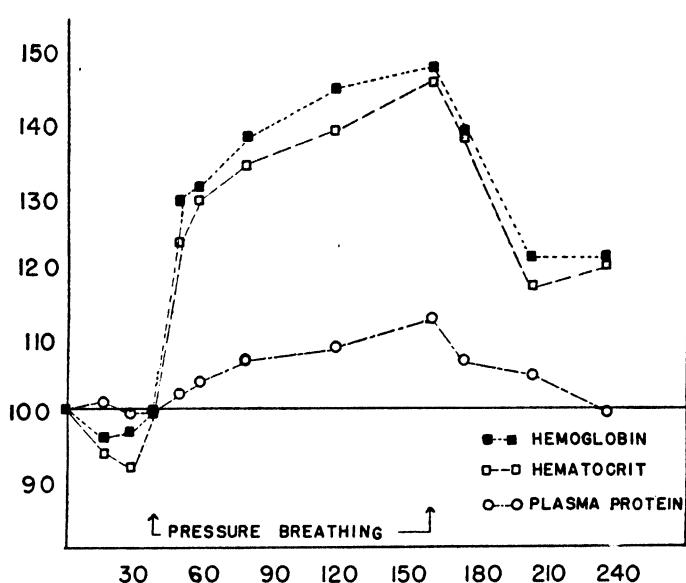
Fig. 3. CHANGES IN HEMATOCRIT, hemoglobin and plasma protein values during an exposure to pressure breathing. Time in minutes, other values as percentage control. Animal which showed marked fluid loss and much protein leakage.
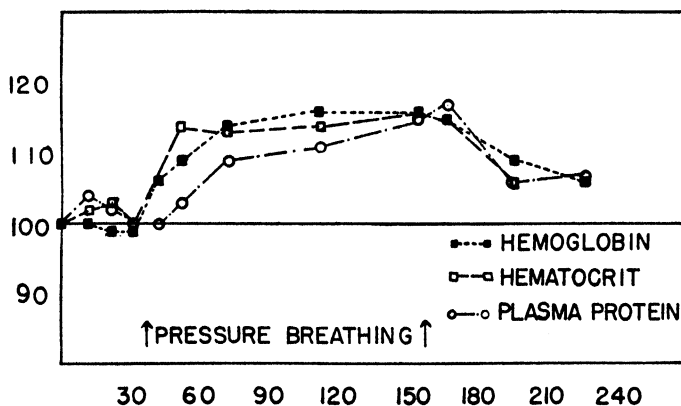
Fig. 4. CHANGES IN HEMATOCRIT, hemoglobin and plasma protein values during an exposure to pressure breathing. Time in minutes, other values as percentage control. Animal with typical fluid loss and no protein leakage.

During pressure breathing, complete counter-pressurization would theoretically protect the subject against all the cardiovascular and mechanical disturbances which otherwise would result from the increased respiratory pressures. With limited counter-pressurization, however, there is opportunity for significant decrease in

effective circulating blood volume due to congestion, pooling and filtration of fluid in the unprotected areas of the body. These mechanisms for fluid loss are merely extensions of the phenomena encountered with pressure breathing at lower levels where counter-pressurization is not employed.

The findings in the present study confirm the observations of Henry, *et al.*, made on man during conscious pressure breathing and extend them to anesthetized animals. Certain of the results, however, are specific for the preparation used and should be considered here.

The hematocrit:protein ratio may reflect the general state of the animal, or may more specifically measure the absolute anemia of the cats. In either event, the ratio serves as an empirical index for predicting which animals will behave in an atypical fashion during pressure breathing. It is unlikely that the anemia itself is of sufficient magnitude to lead to an anemic anoxia with a consequent increase in capillary permeability. Henry, Goodman and Meehan (5) have shown that extremely low oxygen levels are required to produce changes in capillary permeability. These studies again demonstrate the rapidity with which fluid may be displaced from the circulation by purely hydrostatic factors. Shifts of more than 10 per cent of the total blood volume in less than 30 minutes of pressure breathing have frequently been observed.

SUMMARY

A method has been developed for exposing anesthetized animals to high positive pressure respiration, using a counter-pressurization system and a simple helmet. Animals exposed to pressure breathing show a rapid loss of fluid from the circulation which is roughly proportional to the mean respiratory pressure employed. Protein leakage was inconstantly observed in animals exposed to pressure breathing.

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NATURE OF THE PRECORDIAL ELECTROCARDIOGRAM[1]

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THE precordial electrocardiogram has assumed importance in clinical electrocardiography because in certain instances it shows changes not apparent in records from the limb leads, and on this ground alone it would be useful to have more complete information concerning its origin. In addition to the simple recognition of normal and abnormal complexes, the lead has been credited with a special significance in regard to electrocardiographic theory. The essential feature of its special significance lies in the assumption that unipolar precordial leads "are in reality semi-direct leads from the anterior ventricular surface, capable within certain limits of serving the same purposes as direct leads from the ventral wall of the exposed heart" (1, p. 27).

Viewed in this light the lead becomes largely a reflection of physiological events transpiring within the myocardium lying directly beneath the exploring electrode, with lesser contributions from other regions made possible by the alterations they cause in the potential of the cavity, which are thereby transmitted to the exploring electrode.

While therefore unique properties of a theoretical nature are invoked for precordial leads, in practice the records taken with such a lead are interpreted as representing the full sequence of excitation and recovery of all parts of the ventricle beneath the recording electrode. In picturing this chain of events, resort is had to the conventional dipole hypothesis. The upstroke of R is taken to represent the outward passage of the wave of excitation from endocardium to epicardium directly beneath the chest electrode, producing, according to theory, an electrical change which can be represented by a dipole with the positive charge oriented toward the epicardium. The downstroke is inscribed when the full thickness of the ventricle under the electrode has become excited and local differences of potential between endocardium and epicardium disappear. Q-waves are said to appear when some part of the endocardial myocardium at a distance from the chest electrode is excited in advance of the region under the electrode. Such activity would produce a similar array of dipoles in that region and, since normal excitation is considered to progress in all parts of the heart from endocardium to epicardium, the cavity of the ventricle would become negative, and this negativity, being transmitted to the chest electrode, would produce the downstroke of a Q-wave. In a like manner an S-wave would appear if an impulse were still traveling in an outward direction in any part of the ventricle after the impulse had already broken through to the surface underneath the chest electrode.

The T-wave is supposed similarly to arise mainly from differences in the time of onset and rate of repolarization between endocardial and epicardial muscle layers lying beneath the exploring elec-

trode, although similar differences in the repolarization of endocardial and epicardial layers at distant points would also affect the T-wave because of the influence they would exert on the potential of the cavity.

When this hypothesis is used to interpret complexes of unknown origin, it becomes apparent immediately that two alternative explanations are always possible for each portion of the ventricular complex. Thus a Q-wave might represent the outward travel of the excitation process at a point at a distance from the chest electrode or the inward travel of an impulse beneath the precordial electrode. The R-wave could represent the inward travel of a wave of excitation distant from the chest electrode, or an outward traveling wave beneath it. Alternative origins for the S-wave are also possible. It may be granted that some of these alternatives are not likely to occur if one assumes a certain normal sequence of excitation, but they become as likely as any of the others in cases of conduction defects and abnormal rhythms.

The alternatives regarding the T-wave are not however resolved by recourse to assumptions regarding the normal distribution of the cardiac impulse. To explain an upright T-wave it is assumed either that the epicardium beneath the exploring electrode begins repolarization ahead of the subjacent endocardial lamellae, or that the endocardium at a distance begins its repolarization before the overlying epicardium, and there is no collateral evidence to indicate which if any of these is the more likely. The localizing significance of RS-T segment deviations is equally unclear. It has been claimed that lesions damaging the endocardium beneath the precordial electrode produce downward displacement of the RS-T segment, while it is known that similarly situated epicardial lesions cause an upward deviation (2). Thus it would be impossible to determine from an upward displacement of the RS-T segment whether the lesion is in the epicardium directly beneath the electrode or in the endocardium at a distance.

Some direct experimental evidence bearing on these questions is already at hand (3). An R-wave could never be produced by stimulation of the endocardium underneath the chest electrode. Neither could Q- or S-waves be produced by stimulating endocardial areas of the heart at a distance from the electrode. Quite to the contrary, R-waves could only be produced by stimulation of distant areas, and Q- and S-waves were only produced by stimulation of either endocardium or epicardium in the region of the chest electrode (3).

A comparable study of the T-wave in which localized endocardial or epicardial cooling or warming was employed showed that it was not possible to differentiate between the effects of treatment of epicardial and immediately subjacent endocardial layers of the myocardium (4). The only way to invert the T-wave was to delay repolarization of the muscle underneath the chest electrode whether endocardial or epicardial, or to hasten repolarization of distant regions, whether endocardial or epicardial. Similarly increase in amplitude of T could be produced only by hastening repolarization of epicardial or underlying endocardial regions under the electrode or by delaying repolarization of distal regions be they endocardial or epicardial (4).

The implication of this evidence is that the precordial electrocardiogram arises from the interplay of but two opposing forces, one representing the excitation of the proximal region under the chest electrode and the other the excitation of certain regions distal to the chest electrode. Quite without regard to the pathway by which excitation arrives, activation of the proximal zone tends to produce a downward movement in the record, and depending on the sequence of events in the areas involved, produces respectively the downstrokes of Q, R and S. Excitation of distal areas is responsible for the upward movement in the record, whether toward the diastolic baseline as in Q and S, or away from it as in R. These functions are exclusive; downward movements can be produced only by 'proximal' excitation, upward movements only by 'distal' excitation. Stated in another way the evidence thus far produced supports the view that the precordial electrocardiogram is derived from the interference between potentials resulting from excitation in the proximal zone which tend to move the beam downward and potentials derived from excitation of distal regions whose effect is to move the beam upward.

The experiments reported here were devised to extend the observations outlined above, and to delimit in some detail the topographical extent of the distal and proximal zones as recorded in the several precordial leads in the dog's heart.

METHODS

Fifteen dogs were employed, anesthetized deeply with Nembutal or Dial. The heart was exposed by incision along the lower border of the left pectoral muscle and
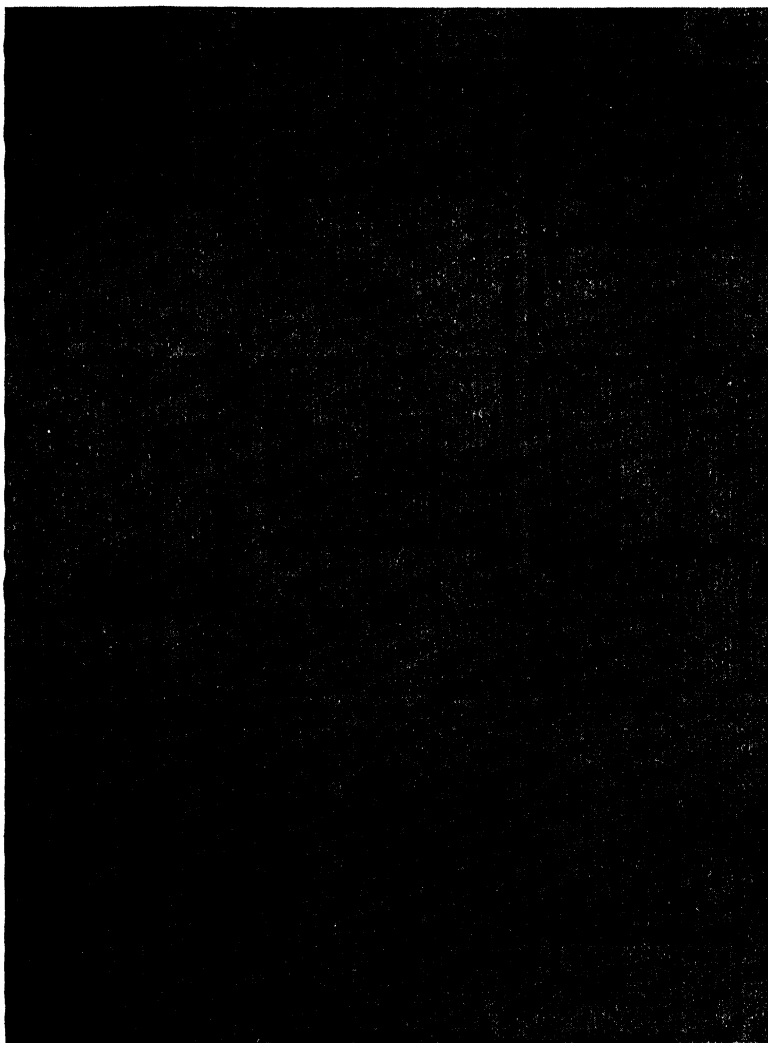
Fig. 1. Dog, Sept. 1, 1944. Dial anesthesia. Right apical precordial lead. 1, CR; 2, CF; 3 CV. *A.* Control. *B.* Warming left base (distal zone). T wave becomes sharply inverted. *C.* Warming right apex (proximal zone). T wave sharply upright.

removal of the sternal third of the fifth rib. Lungs were inflated fully, the chest wall was closed and spontaneous respiration reestablished before records were taken, except in experiments with warming or cooling, where a completely air-tight closure could not be effected. CF, CR and CV leads were employed with the chest electrode

opposite the left apex on the left side of the chest and the right apex on the right side of the chest. A Sanborn Tribeam was employed at standard sensitivity. The entire external surface of the heart was explored to determine *a*) the influence on the S-T segment of localized application of o.1 M KCl solution by means of squares of blotting paper soaked in the solution, *b*) the influence of local warming and cooling
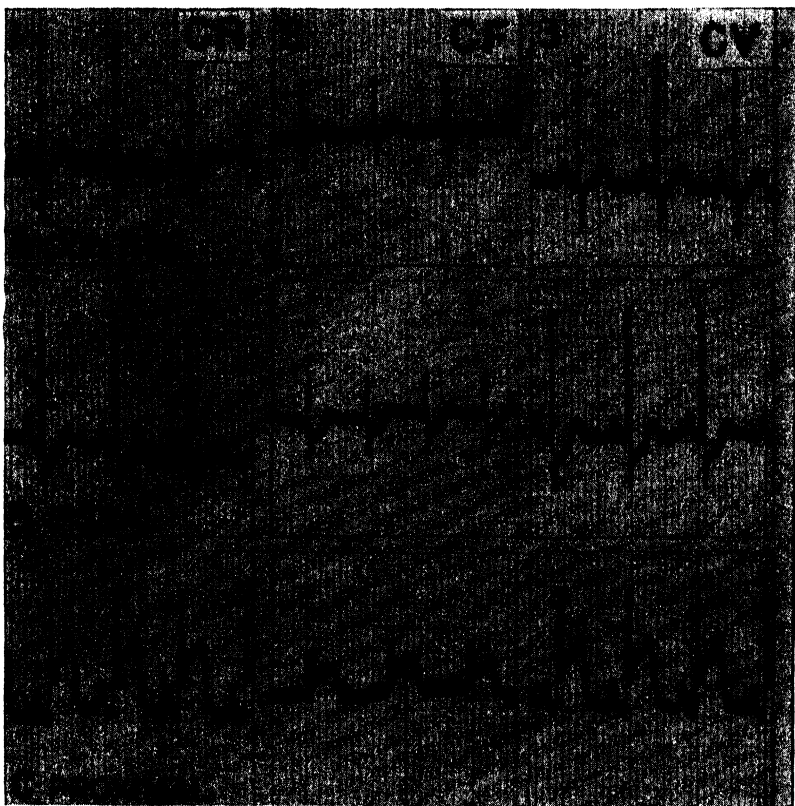
Fig. 2. DOG, same as fig. 1. Right apical lead. *A*. Control. 1, CR; 2, CF; 3, CV. *B*. Isotonic KCl applied to right base (distal zone). S-T segment depressed in each lead. *C*. Isotonic KCl applied to right apex (proximal zone). S-T segment elevated.

of the myocardium on the T-wave and *c*) the configuration of extrasystoles elicited from the regions of the heart studied in (*a*) and (*b*).

RESULTS

All three modes of investigation yielded comparable results: 1) in each case an area could be mapped out under the chest electrode which, when warmed, damaged, or stimulated, produced characteristic 'proximal' electrocardiographic changes. 2) An area of considerably greater extent involving regions of the heart distant from the chest electrode could be demarcated which gave rise to alterations of opposite

nature to the 'proximal' changes; these can be called 'distal' effects. 3) Between these regions was found an intermediate zone which was largely silent or neutral.
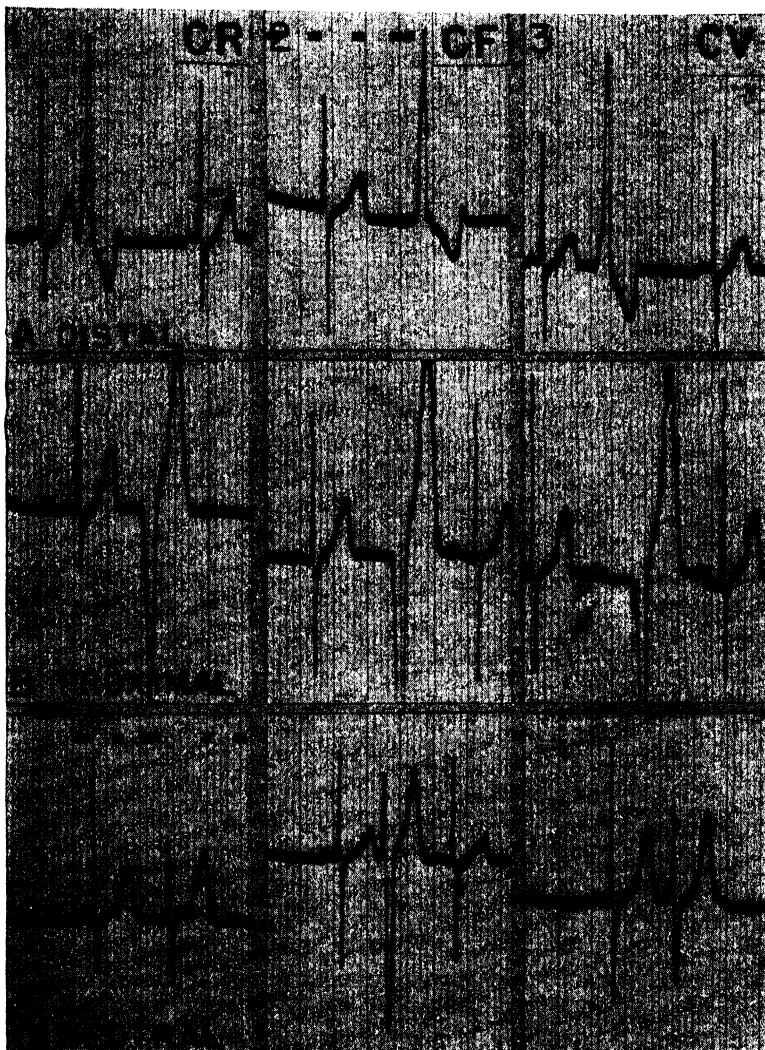
Fig. 3. Dog, August 31, 1944. Dial anesthesia. Right apical lead. *A*. Extrasystoles in CR, CF, and CV leads elicited by threshold stimulation of a point in the anterior left base (distal zone). Initial beam movement is upward. *B*. Extrasystoles elicited by threshold stimulation of a point at the right apex at the septum (proximal zone). Initial beam movement is downward. *C*. Extrasystoles elicited by threshold stimulation points in the neutral zone of each lead. 1. left lateral apex; 2. right base; 3. posterior septum midway between base and apex. In each case a diphasic complex results.

Localized warming of the myocardium under the chest electrode caused a progressive elevation of the T-wave leading to the development of exaggerated positive

T-waves. These reached a maximum height after one or two minutes and there-after maintained that amplitude for as long as warming persisted. Warming distal regions produced, on the contrary, a progressive diminution in height of the T-wave
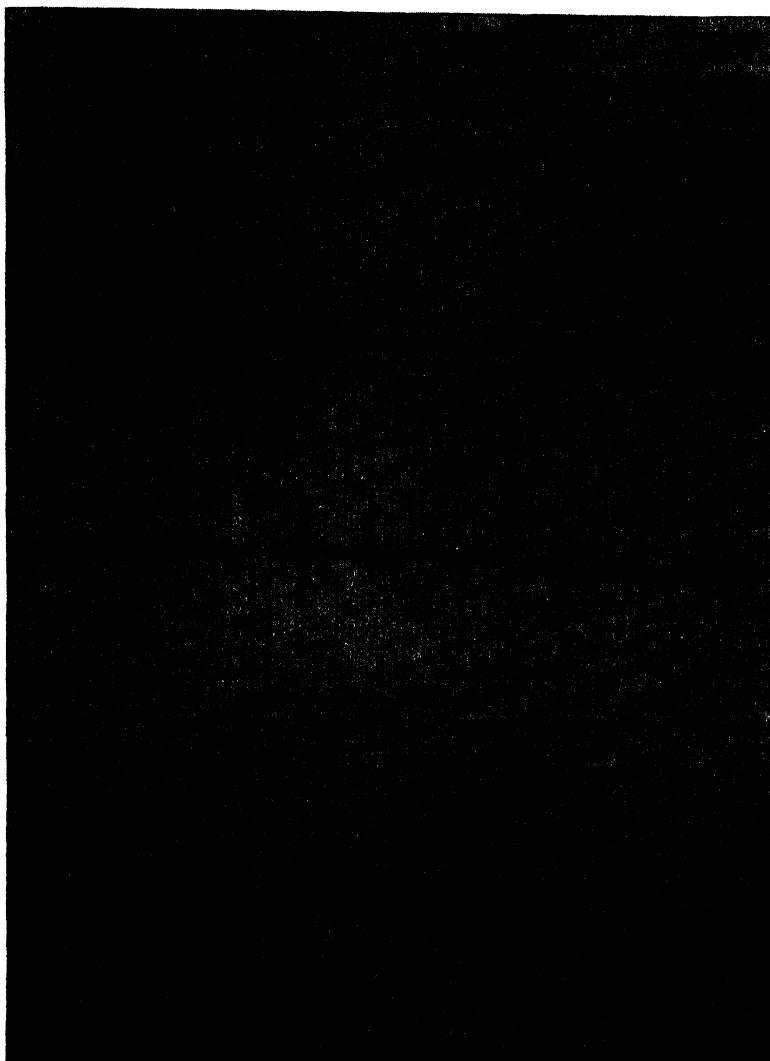
Fig. 4. SAME EXPERIMENT as fig. 1. Left apical precordial lead. *A*. Control. 1, CR; 2, CF; 3, CV. *B*. Warming posterior base (distal zone). T-wave becomes sharply inverted. *C*. Warming left apex (proximal zone). T sharply upright.

culminating in inversion. Similar treatment of intermediate areas was without influence on the T-wave. Cooling the proximal zones caused inversion of the T-wave while cooling the distal zone caused elevation of the T-wave (figs. 1, 4).

The reversible damage caused by application of 0.10 M KCl solution to the proximal area under the chest electrode always evoked an upward displacement of the RS-T segment, while damage to distal areas was always followed by a depression of the RS-T segment.   Intermediate silent areas again separated distal and proximal zones (figs. 2, 5).
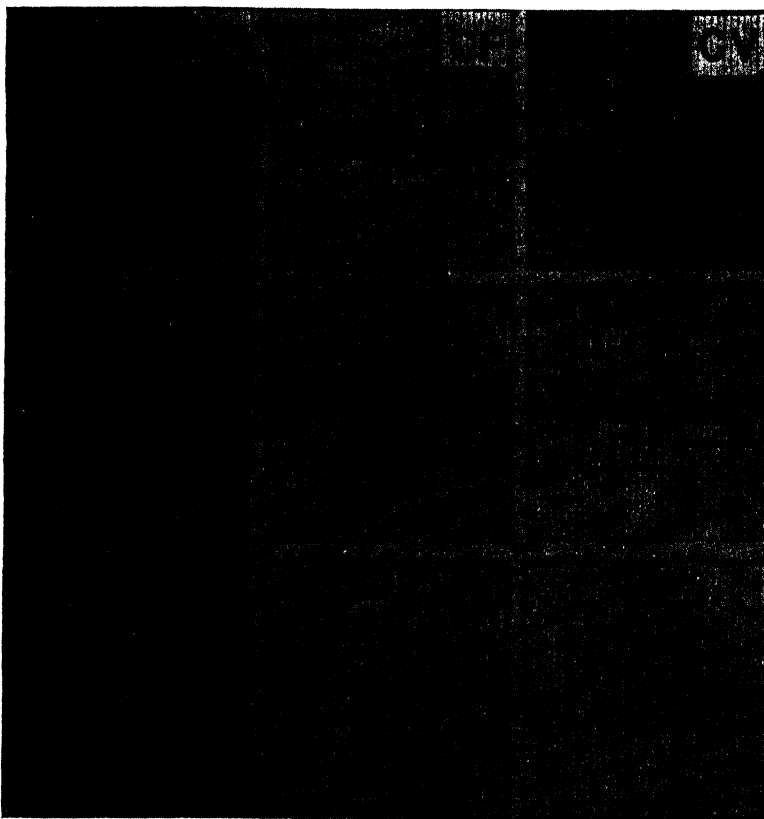
Fig. 5. Dog, same as fig. 1.   Left apical lead.   *A*. Control.   1, CR; 2, CF; 3, CV.   *B*. Isotonic KCl applied to posterior base (distal zone).   *C*. Isotonic KCl applied to left apex (proximal zone).   S-T segment elevated in each lead.

Extrasystoles elicited from proximal areas invariably exhibited only a simple QS configuration, while those arising in distal areas showed only an R-wave (figs. 3, 6).   Intermediate zones gave rise to QRS patterns in which R became more prominent as the point of stimulation approached the distal zone, while S was augmented as the proximal zone was approached.

With each position of the chest electrode, the proximal zone always included at least the apex of the ventricle corresponding to the side of the chest lead selected, while the distal zone always included some portions of the base of the heart.   In other words, the chest leads always included an element of base-apex interference.

In general, the complexes were of greater amplitude and the extent of the proximal zone was less in the CR and CF leads than with CV leads.   From the standpoint of sharpness of delimitation of the proximal area, therefore, leads CR and CF were
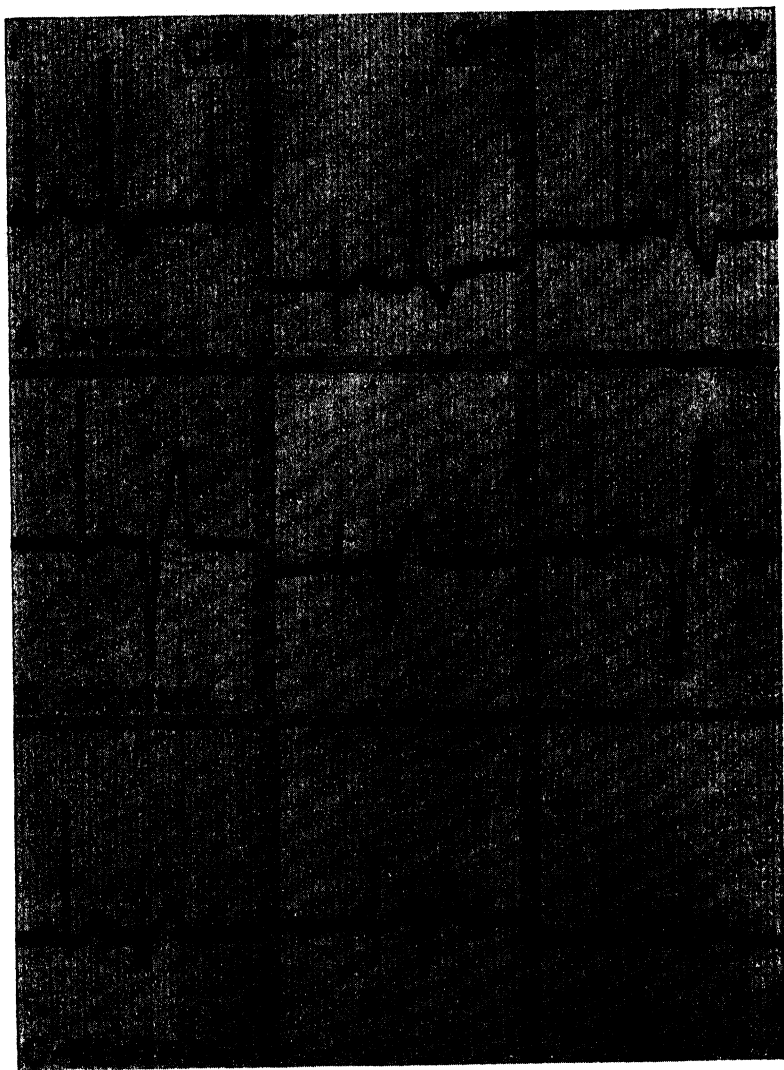
Fig. 6. Doo, August 31, 1944.   Left apical lead.   *A*. Extrasystoles by threshold stimulation of a point in the distal zone.   1, CR; 2, CF; 3, CV.   Initial movement of the beam upward.   *B*. Extrasystoles elicited by threshold stimulations of a point in the proximal zone.   Initial beam movement downward.   *C*. Extrasystoles elicited by threshold stimulation of a point in the neutral zone. Multiphasic QRS.

slightly superior to CV.   In all leads studied the proximal and distal zones as mapped out by the extrasystole method could be defined more exactly than by the method of warming and cooling or local surface damage.   Figures 7 and 8 summarize the data obtained in the various experiments for each lead.

CR   CF   CV
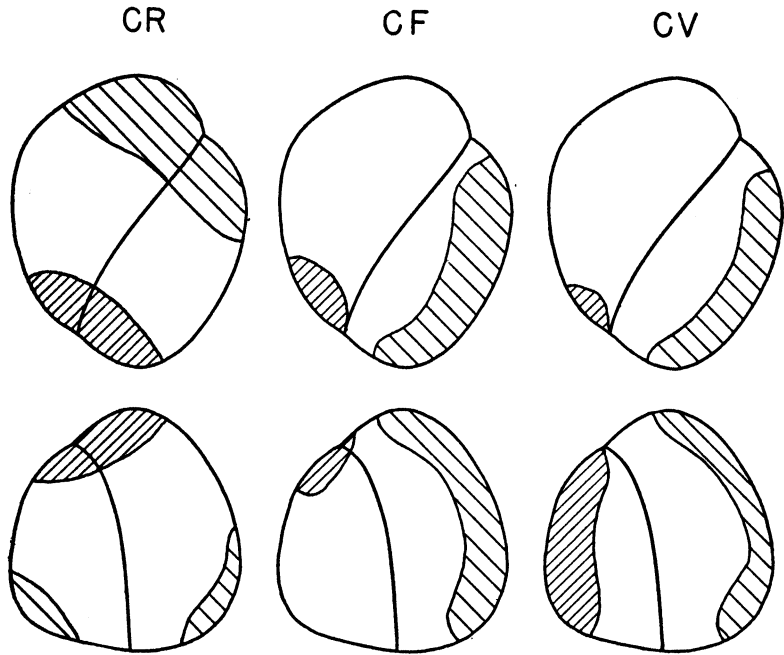
[Fig. 7. SUMMARY of the proximal and distal zones of the dog heart when the lead is taken with the exploring electrode placed over the right apex; CR, CF, and CV leads. *Above*, ventral surface of the heart, apex down; *below*, dorsal surface of the heart, apex up. *Fine shading*, proximal zones; *coarse shading*, distal zones.

CR   CF   CV
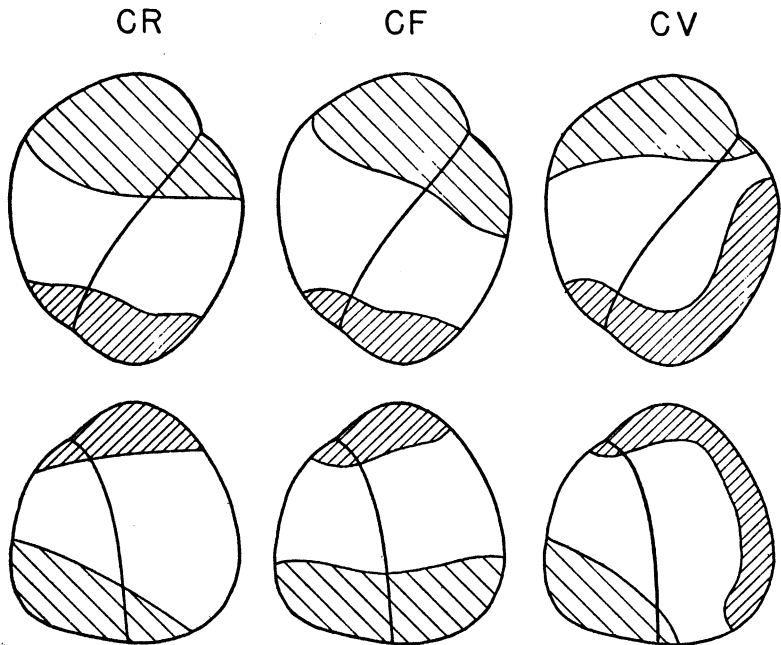
Fig. 8. SUMMARY of proximal and distal zones of the dog heart when the lead is taken with exploring electrode over the left apex. *Fine shading*, proximal zones; *coarse shading*, distal zones.

DISCUSSION

When these experiments are considered in the light of the failure to establish any differences in the contribution of epicardial and immediately subjacent endocardial surfaces of the myocardium to the electrocardiogram, the conclusion appears justified that each precordial electrocardiogram reflects the interplay of two forces arising from the excitation and recovery of specific proximal and distal zones of the heart with respect to the position of the chest electrode.

The only force that can produce a downward movement in the QRS portion of the electrocardiogram is derived from excitation of the myocardium lying beneath and in close proximity to the chest electrode (the proximal zone). The direction from which the wave of excitation approaches makes no difference whatever, and in particular it is a matter of indifference whether the endocardial or epicardial surface of the myocardium is the first to be excited. Only excitation in the proximal zone can evoke a downward movement in the electrocardiogram, and by no means is it possible to produce such a movement by initial activation of the distal zone. If the region underneath the chest electrode is the first of all parts of the heart to be fully excited, a Q-wave or QS complex is produced, while the prior activation of a distal area is responsible for the appearance of an R-wave. The downstrokes of R and of S are also the result of proximal excitation, while the upstrokes of Q, R, and S indicate distal excitation. Reference to figures 6 and 7 shows that the areas involved in the proximal as well as distal zones are large enough to encompass both early and late subdivisions, and these account for various combinations of Q, R and S.

The T-wave is also formed from the summation of the electrical effects of recovery in proximal and distal areas. An initial upward movement indicates beginning recovery in the proximal zone, and downward movement indicates beginning recovery in the distal region. An upright T-wave develops when the distal region recovers later than the proximal zone, while an inverted T-wave results when the proximal zone recovers later than the distal zone.

The depression or elevation of the RS-T segment that develops with the establishment of localized areas of injury fits into the same pattern. Surface injury in a proximal zone produces elevation of the S-T segment, while surface injury in the distal zone produces a depressed RS-T segment. Surface injury in the intermediate zone caused no deviation in the RS-T segment.

The chest leads differ from the standard leads only in that the areas involved in the interference are unequal in size and are differently located, tending at times to have base-apex localization. The apex of the ventricle underneath the chest electrode was always included in the proximal area, as was also the apex of the opposite ventricle in some instances. Less frequently the proximal zone extended in a narrow projection toward the base, either anteriorly or posteriorly. The proximal zone was also considerably smaller than the distal zone, although this feature was less marked when CV leads were employed. The distal zone in these apical leads always included portions of the base, especially the lateral portions as its most important elements, and only infrequently were projections noted toward the apex of the ventricle opposite that under the recording electrode.

It is of course to be remembered that these experiments were carried out on the heart of the dog, and the particular topography of distal and proximal zones cannot be applied exactly to the human heart. A chest lead, taken from any point, will have its own particular zones of interference, depending upon the position of the electrode as well as the size and orientation of the heart. There seems no reason to doubt that the subdivision of the heart into two major interfering zones separated by a neutral area exists for the precordial lead of man as it does in the dog. There is furthermore no reason to believe that the proximal zone is proportionately any less extensive in man than in the dog.

SUMMARY

1. The precordial electrocardiogram represents the interference of opposing electrical forces developing with the excitation and recovery of specific regions which are proximal and distal with respect to the position of chest electrode. An intermediate zone rather large in extent separates the proximal and distal zones of each lead. Potentials derived from the excitation of this zone fail to be reflected in the precordial electrocardiogram.

2. The downstrokes of Q, R, and S are caused by preponderance of excitation in proximal areas. The upstrokes of Q, R, and S are caused by preponderance of excitation in distal areas.

3. The T-wave is similarly derived from differences in the time and rate of recovery from excitation in distal and proximal zones. An upright T-wave indicates beginning of repolarization in the proximal zone in advance of the distal region, while an inverted T indicates that regions in the distal zone begin to repolarize in advance of the proximal zone. The S-T segment is elevated when injury is present in the proximal zone, and depressed when injury occurs in the distal area.

4. Injury to the large intermediate zone cannot be detected in the precordial electrocardiogram. The proximal and distal zones for three different leads (CR, CF and CV) were determined in the dog when the chest electrode was placed external to the left and right apex.

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DETERMINATION OF CIRCULATING RED BLOOD CELL VOLUME WITH RADIOACTIVE PHOSPHORUS[1]

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D IRECT measurement of total circulating red blood cell volume has been accomplished most accurately in man by an isotope dilution technic using red blood cells labelled with radioactive iron (1, 2). The method requires an available and previously prepared donor with labelled red cells and considerable technical skill in the handling of blood samples submitted for analysis. It is not a method that can be adapted easily to wide clinical exploitation.

This report has two purposes: first, to present a method for measuring the total circulating red blood cell volume in man by an isotope dilution technic using radioactive phosphorus; and second, to discuss sources of error in the proposed method.

The method is a modification of that proposed and used by Hahn and Hevesey (3), Anderson (4), and Brown *et al.* (5). It has many advantages, chiefly those of simplicity in the labelling of red blood cells and in the preparation of samples for counting. In all instances the blood of the subject to be studied has been used for labelling.

METHOD

Labelling of Red Blood Cells. Ten (10) ml. of blood was withdrawn under aseptic precautions from the subject into a sterile heparinized syringe and transferred to a sterile centrifuge tube containing 50 microcuries of P-32 as phosphoric acid (H_3PO_4) in 0.1 to 1 ml. of autoclaved solution. This mixture was incubated at 37°C., in a water bath, for 2 hours with mild manual agitation every 20 minutes (approximately 40% of the P-32 was taken up by red blood corpuscles during this period)[2].

The mixture was then centrifuged and the supernatant discarded. The cells were resuspended in 8 to 10 ml. saline, washed by gentle agitation, and the mixture was centrifuged and the supernatant discarded. The cells were finally resuspended in saline to a total volume of 10 ml. and thoroughly mixed. This was the final suspension of labelled red blood cells. It was used in part for injection into the sub-

[2] We have also used the procedure of Anderson (4) of bubbling 5% CO_2 and 95% O_2 through the blood sample. This increases the rate of uptake of P-32 by the cells and shortens the incubation period considerably. The results given in this and the subsequent papers have, however, been obtained by the procedure described above.

ject of study and in part to obtain a count of the radioactivity of the labelled red blood cells.

Injection of Labelled Red Blood Cells and Sampling After Injection. An exactly measured quantity, usually 5 ml., of the final suspension of labelled red blood cells was taken up in a calibrated syringe. This was injected directly into an antecubital vein, care being taken to ensure complete delivery by aspiration and injection of blood to wash the syringe at least twice. After a lapse of 10 minutes, 4 to 6 ml. of blood were withdrawn from the opposite antecubital vein without a tourniquet into a heparinized syringe and transferred to a glass container.

Handling of Blood Samples. Two sources of blood were utilized: *a*) From the final suspension of labelled red blood cells, as prepared for intravenous injection, 1 ml. was added to a volumetric 50-ml. flask and made up to a volume of 50 ml. with tap water. Duplicate dilutions were made. The cells were laked during this process. One ml. of each of these laked 1:50 dilutions of the final suspension of labelled cells was added to each of 2 sample dishes for counting. *b*) From the heparinized whole blood withdrawn from the subject after mixing of the injected labelled cells, two determinations were prepared: 1) one ml. was added to each of two sample dishes, 2) a Wintrobe hematocrit tube was filled and centrifuged at 3000 r.p.m. (checked by tachometer) for 30 minutes. The radial distance from the axis of the centrifuge to the bottom of the tube was 20 cm. The observed red cell concentration was reduced by 8.5 per cent to correct for the quantity of plasma retained among the cells (6).

Assay of Radioactivity of Samples. *a*) *Preparation of sample dishes.* The sample dishes were prepared from $\frac{1}{4}$-ounce ointment tins (bottoms only). Filter paper discs were prepared to fit exactly into the tin. Rubber cement was spread in a thin layer on the tin and on one surface of the filter paper and allowed to dry for two to three minutes. The filter paper disc was cemented into the tin and allowed to dry at room temperature.

b) *Drying of samples.* The blood samples to be counted were allowed to run onto the filter paper. This assured even distribution. The samples were then dried without boiling, which is important. Drying is done most expeditiously in an oven at 60° to 80°C.

c) *Counting of samples.* The counter tube was arranged in a brass box with a sample-holder that fit exactly the ointment tin and ensured a uniform geometry of sample and counter. Variations of surface geometry were minimized by even distribution of the added blood, but duplicate samples reduced the error from this source to a ±2 per cent. The counting was done with a thin glass walled, cylindrical beta counter.

Calculations. The counts per second of duplicate samples were averaged. Counts per second of diluted samples were recalculated to counts/sec/ml. of injected suspension. It was assumed that the counts per second of whole blood, as determined, represented the activity of the previously injected cells. The quantity of cells actually present in 1 ml. of the whole blood sample was determined as described above. The counts per second of a known quantity of cells were thus obtained. From this value, the counts per second of 1 ml. of cells was calculated.

The total circulating volume of red blood cells was calculated from the following formula:

$$\text{mls. of red blood cells} = \frac{\text{Total counts/sec. injected}}{\text{Counts/sec./ml. of cells withdrawn}}.$$

SOURCES OF ERROR

During the developmental experience with this method, a number of controversial issues and apparent sources of error were visualized. Certain of these problems are considered below:

Loss of P-32 from Labelled Cells. The most likely source of serious error in the method is the possible loss of P-32 from the red cells to plasma and extravascular compartments, which would appear as excess dilution and give values for red cell volume that would be too high. Both *in vitro* and *in vivo* studies of such loss have been made.

Labelled cells have been repeatedly washed with both normal saline and unlabelled plasma, and the increase in activity of the washing observed. This increase is variable, but averages about 10 per cent of the total initial activity of the centrifuged cells. Most of the increase occurs within the first 10 minutes of the first washing. In subsequent washings only a small increase, exponential with time and dependent upon the volume of solution is observed. In large dilutions (1 to 1000 for red cells to saline) this amounts to less than 3 per cent of the activity of the cells.

There is reason to believe (6) that some plasma remains with the cells as a contaminant after the separatory centrifugation, and amounts to 8 or 9 per cent of the red cell volume. Since the P-32 content of the plasma from which the labelled cells was separated varies from 80 to 150 per cent of the content of the cells, an activity of 6 to 12 per cent of the total activity of centrifuged cells plus contaminant would be expected to appear in any washing solution which removed or diluted the contaminating plasma. This amount of activity is not truly present in red cells at any time before injection into a subject and is largely, but not completely, removed by the single washing employed in routine applications of the method. All of the true loss of activity from cells to solution, corresponding to the second observation above, and the activity of the residual labelled plasma carried over after the single washing is regarded as belonging to the cells of the suspension prepared for injection.[1]

The justification for this assumption appears in the results of two types of *in vivo* experiments. The first type of experiment was made possible through the use of large intravenous doses of P-32 for therapy. Here the loss of P-32 from the blood stream to the extravascular compartments and from plasma to red cells could be followed conveniently.

The results are represented in figure 1. The rate of loss from whole blood is shown to be approximately one-half the rate of loss from plasma. The difference between the loss from plasma and the loss from whole blood represents the transfer of active material from the plasma to the initially unlabelled red cells. Therefore, only half the P-32 in the plasma is assumed to leave the plasma to go into the extravascular compartments. The other half of it goes into red cells. If, therefore, an injected sample of labelled red cells either carries some contaminated plasma

along—or loses P-32 to the plasma after injection—only one half of it will be lost to extravascular compartments, the other half would return to more red cells. The 'loss' observed in previous experiments amounted to a maximum of 10 per cent; half of that we now concede lost to extravascular compartments; we are left with a 5 per cent loss which agrees well with the loss observed in successive samples as described below. Reeve and Veall (7) report a mean loss of about 6 per cent during the first hour after injection, but on occasion the loss appears to be negligible.

In the second type of experiment, blood samples are taken at intervals after the injection of labelled cells and the activities compared. Over long ranges of time, from one hour to 48 hours, the disappearance of the labelled material is remarkably
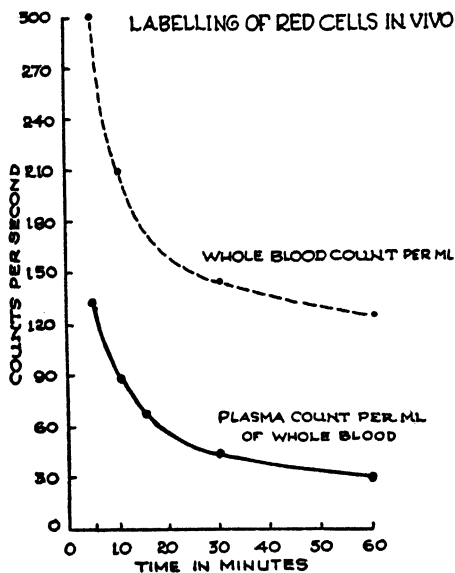
Fig. 1

constant from one subject to another, the average rate of loss being such that after the elapse of 24 hours, 47 ±2 per cent of the activity remains in the red cells. Extrapolation from the long-range (1 to 24 hours) disappearance curves gives a loss over the first 15 minutes of 2 per cent. The curve satisfies the relation, $\log A_t - \log A_0 = kt$ where A_t and A_0 are the activities at time t and o respectively, k is a constant, and t is elapsed time. This indicates that the time loss of activity is small and constant.

However, when observations are confined to intervals of 5, 10, 15 and 30 minutes, no such smooth curve is observed and the apparent loss is much greater and variable as shown by the data in table 1. Even here, the average loss is not greater than 5 per cent and the extreme cases are included in this small selection of the data.

These variations in apparent active phosphorus concentration in the first 30 minutes after injection as compared with the smooth disappearance curve that is obtained over longer intervals suggests that other factors than loss of P-32 are at

work immediately after injection of the labelled cells. Nevertheless, the data may be taken as indicating an average loss of 5 per cent of the injected label over the time required for the volume determination.

Errors of Self-absorption and Geometry in Counting. Errors of self-absorption and geometry in counting were studied by making duplicate determinations of samples made up to different dilutions ranging from 1:1 to 1:1000. The standard error attributable to such sources was less than 2 per cent. Counts were made to an accuracy better than 1 per cent.

TABLE 1. PERCENTAGE OF ORIGINAL ACTIVITY (OF FIRST SAMPLE DRAWN) REMAINING AFTER VARIOUS INTERVALS

EXP. NO.	MINUTES AFTER INJECTION				
	5	10	15	20	30
1		100		102	
2	100		101	102	
3		100	97		
4	100	96	89		
5		100	99		
6	100	97			
7	100	98	97		
8	100	98		95	
9	100	86		77	
10		100		96	92
11		100		102	

TABLE 2. RESULTS OF DUPLICATE DETERMINATIONS OF RED CELL VOLUME

DISCREPANCY	NO. OF CASES
0– 2%...	10
2– 5%...	8
5–10%...	1

Duplicate Determinations to Check Overall Consistency. A summary of the discrepancies observed between maximum and minimum values obtained in 19 sets of duplicate determinations of red cell volume is presented in table 2.

OBSERVATIONS

Measurements of total circulating red blood cell volume were made in a large number of healthy individuals and patients suffering from a variety of clinical disabilities. Some of these results are presented in the papers which follow. The results are in close agreement with similar measurements made by us and by others with different technics.

Clinical experience with the P-32 method has demonstrated its utility, particularly under circumstances where repeated determinations are desired. The activity of the tagged cells is $\frac{1}{10}$ of the safe tolerance dose and 75 per cent excretion occurs

in 48 hours.   For repeated determinations, therefore, only a small correction need be made for the residual activity of the patient's blood.   This may be determined from the blood sample withdrawn for labelling.

SUMMARY

A method for the direct measurement of total circulating red blood cell volume by an isotope dilution technic using radioactive phosphorus has been presented.   The red cells from the subject of study are utilized for labelling.   Rapid uptake and slow release of radioactive phosphorus by exposed red cells facilitates wide experimental application.   Ease of counting and the opportunity for repetitive measurement are other advantages.

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COMPARISON OF RESULTS OF MEASUREMENT OF RED BLOOD CELL VOLUME BY DIRECT AND INDIRECT TECHNICS[1]

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CONSIDERABLE controversy has arisen over discrepancies in the volume of circulating red blood cells in accordance with variations in the circumstances or methods of measurement (1–4). In general, the total circulating red blood cell mass is smaller when measured directly (radioactive iron technic, carbon monoxide method) than when calculated from the plasma-dye-hematocrit observations (T-1824 technic). We have made certain observations upon this discrepancy as a consequence of concomitant measurement of the red cell mass with the P-32 technic described in the preceding paper and of plasma volume with the T-1824 method as described by Gregerson (5).

Eighty sets of determinations were made upon 10 'normal' individuals and 35 patients hospitalized for various reasons. Determinations were made under basal conditions. Individuals in usual health omitted breakfast and rested in the laboratory for one-half hour before the test was performed. Hospitalized patients were allowed to remain quietly in bed without breakfast. The standard procedure was to withdraw 10 cc. of blood from an antecubital vein without stasis and to inject through the same needle 5 cc. of red cells tagged with P-32 (as described in the preceding paper). The syringe was rinsed twice with blood and 5 cc. of T-1824 solution was injected and the syringe rinsed twice again. After 10 minutes, 15 cc. of blood was withdrawn from the opposite antecubital vein and the sample divided for the determination of the red cell and plasma volumes.

In assessing the validity of the red cell volume as estimated from the plasma-dye-hematocrit values, two points are crucial. 1) The first relates to the accuracy of the *in vitro* determination of the hematocrit in peripheral venous blood. The usual value given for the peripheral venous hematocrit ignores plasma trapped between sedimented red blood cells in spite of the recognized existence of this source of error. Reluctance to correct for this error stems from uncertainty as to the correction factor to be applied. Chapin and Ross (6) have determined this factor as 8.5 per cent. Using similar methods, we have corroborated this value as applicable to the hematocrit determined by centrifuging in Wintrobe tubes at 3000 r.p.m. for 30 minutes. The distance from the axis of the centrifuge to the bottom of the tube is 20.0 cm. Correction on this basis has become standard practice in our laboratory.

2) The second point relates to the identity of the *in vitro* measurement of the

peripheral venous hematocrit with the ratio of cells to plasma in the entire vascular bed, the 'body hematocrit'. Pertinent data on this issue can be obtained only by concomitant measurement of peripheral venous hematocrit, direct measurement of plasma volume and direct measurement of total red blood cell mass.

RESULTS

Typical results obtained by simultaneously measuring the blood volumes of healthy and sick individuals with P-32 and T-1824 are given in table 1. The hematocrit values used in all of the calculations are the observed hematocrits corrected by the factor 0.915.

Comparison of the red cell volumes as obtained by the plasma-dye-hematocrit and the P-32 methods show a ratio of 0.96 for the entire series of 26 cases. All of the 6 cases in the normal group and ten of the 12 cases in the male hospitalized group have values which agree to within 10 per cent. Eleven of these 18 cases agree to within 6 per cent. There is a marked discrepancy in only one case (no. *16*), in which a biliary fistula was present. The group of female patients show a somewhat greater range than do the other two groups, and the values obtained by the P-32 method are generally lower than those calculated from the plasma-dye method. All of the values, however, agree to within 15 per cent with the exception of *case 24*, a gravely ill patient, where the discrepancy is large (23%).

Ross *et al.* (7) have suggested the use of a second correction factor of 15 per cent to compensate for the error introduced by the unequal distribution of erythrocytes and plasma throughout the vascular system. They claim that the cell volumes of normal human subjects determined in this fashion compare very closely with the cell volume determined in the same subjects with the use of radioactive tagged cells. In table 1, we have applied this correction to our data (RVD $\times$ 0.85) and compared the values so obtained with those obtained by use of the P-32 method $\left(\dfrac{RVP}{RVD \times 0.85} \right)$.

Except for 6 of the female patients who showed low P-32 values as compared to the T-1824 values, the correction introduces a discrepancy approximately equivalent to the correction factor. The reason for the difference in these female patients is not obvious. As a group they were more seriously ill than the male group. On the other hand, several of the male patients (*cases 12* and *14*) were also in critical condition at the time they were studied but showed good agreement in their P-32 and T-1824 values. The number of cases is obviously too small for critical evaluation.

Of particular interest is the remarkably close agreement of the whole blood volumes as calculated from the plasma-dye-hematocrit values (TVD) and the sum of the plasma and the red cell volumes as determined directly by the T-1824 and P-32 methods (TBV). The latter value for whole blood volume is obviously the most accurate estimation since each component is measured directly and independently. Twenty-four of the 26 sets of values agree to within 5 per cent of each other. The two cases in which larger errors were found are *cases 16* and *24* which showed large discrepancies in their red cell and plasma volumes. In spite of these obviously aberrant values, the ratio for the entire group is 0.99. Comparison of the whole blood volumes as calculated from the P-32 data and the hematocrit values yields

TABLE 1

CASE	SEX	WT., KG.	DIAGNOSIS	PVD	PVP	RVP	RVD	$\frac{RVP}{RVD}$	RVD × 0.85	$\frac{RVP}{RVD \times 0.85}$	TBV	TVD	TVP	$\frac{TBV}{TVD}$	$\frac{TBV}{TVP}$	PHC	BH	$\frac{BH}{PHC}$
				PLASMA VOLUME ML.			RED CELL VOLUME ML.				TOTAL BLOOD VOLUME ML.					HCT P.C.		
1	♂	82.0	Normal	3580	3610	2937	2900	1.01	2460	1.19	6517	6480	6547	1.01	1.00	45.1	44.8	0.99
2	♂	60.9	"	2780	2655	2065	2170	0.95	1750	1.18	4845	4950	4720	0.98	1.02	43.9	42.7	0.97
3	♂	61.0	"	2970	2840	1760	1830	0.96	1556	1.13	4730	4800	4600	0.98	1.03	38.2	37.2	0.97
4	♂	60.9	"	2740	2720	1976	1990	1.00	1690	1.17	4736	4730	4666	1.00	1.00	42.1	41.9	1.00
5	♀	53.2	"	2680	2380	1336	1490	0.90	1270	1.05	4016	4170	3716	0.96	1.08	35.6	33.3	0.93
6	♀	57.0	"	2320	2180	1304	1385	0.94	1180	1.11	3624	3705	3484	0.98	1.04	37.5	35.9	0.96
Av. normals				2845	2731	1896	1961	0.96	1651	1.14	4745	4866	4627	0.99	1.03	40.4	39.3	0.97
7	♂	84.5	T. B. arthritis	3270	3060	2280	2430	0.93	2060	1.11	5550	5700	5340	0.97	1.10	42.6	40.7	0.96
8	♂	59.0	Gastrectomy	3320	2840	1912	2210	0.86	1880	1.02	5232	5530	4752	0.95	1.02	40.3	36.5	0.91
9	♂	87.7	Duodenal ulcer	2980	2980	2471	2420	1.01	2060	1.20	5451	5400	5451	1.01	1.00	45.3	45.3	1.00
10	♂	94.1	Polycythemia	2430	2640	2780	2550	1.09	2170	1.37	5210	4980	5420	1.05	0.96	51.2	55.1	1.08
11	♂	63.2	Osteo. sarc.	2980	2980	2004	2000	1.00	1705	1.18	4984	4980	4984	1.00	1.00	40.3	40.2	1.00
12	♂	68.2	Gastric carc.	3680	3660	1084	1090	1.00	930	1.17	4764	4770	4744	1.00	1.00	22.9	22.7	1.00
13	♂	72.7	Osteo. sarc.	2550	2650	1473	1410	1.04	1190	1.24	4023	3960	4123	1.01	0.99	35.7	36.7	1.03
14	♂	73.6	Retic. cell sarc.	2890	3158	842	766	1.09	650	1.29	3732	3746	4000	1.00	0.93	21.0	22.6	1.08
15	♂	68.2	Hepatitis	4100	4455	2145	1970	1.08	1670	1.28	6245	6070	6600	1.03	0.94	32.5	34.4	1.06
16	♂	57.0	Biliary fist.	2340	3080	1790	1350	1.32	1145	1.56	4130	3690	4870	1.12	0.84	36.6	43.8	1.19
17	♂	71.4	Duodenal ulcer	3060	3765	1475	1570	0.95	1335	1.11	5435	5530	5240	0.99	1.04	28.3	27.2	0.96
18	♂	60.9	Hepatitis	2500	2380	1766	1850	0.95	1570	1.11	4266	4350	4146	0.98	1.03	42.5	41.5	0.98
Av. male patients				3083	3137	1835	1801	1.03	1530	1.22	4919	4892	4973	1.01	0.99	36.6	37.2	1.02
19	♀	76.0	Hemolytic anemia	3760	3222	958	1120	0.86	950	1.01	4718	4880	4180	0.97	1.13	20.3	20.3	0.90
20	♀	57.7	Cholecystitis	2650	2620	1400	1410	1.00	1200	1.17	4050	4060	4020	1.00	1.00	34.8	34.6	1.00
21	♀	55.5	Rheumatic arth.	2590	2390	1220	1320	0.92	1120	1.09	3810	3910	3610	0.98	1.05	33.9	31.9	0.94
22	♀	51.7	Anemia	2340	2010	1116	1300	0.86	1100	1.01	3456	3640	3162	0.95	1.08	35.7	32.4	0.91
23	♀	67.6	Breast carc.	2180	1880	1150	1310	0.87	1110	1.03	3330	3490	3030	0.96	1.09	37.6	34.6	0.92
24	♀	41.5	Carcinomatosis	2120	1627	942	1220	0.77	1035	0.91	3026	3340	2569	0.91	1.15	36.6	30.7	0.84
25	♀	66.6	Intest. fist.	3440	2930	1390	1640	0.85	1390	1.00	4830	5080	4320	0.95	1.10	32.2	28.8	0.89
26	♀	65.9	Breast carc.	2425	2115	1295	1515	0.85	1290	1.00	3720	3940	3410	0.95	1.09	38.0	34.0	0.90
Av. female patients				2688	2349	1184	1354	0.87	1149	1.03	3868	4042	3538	0.96	1.08	34.0	30.9	0.91
Av. all subjects				2907	2801	1649	1701	0.96	1441	1.14	4555	4609	4451	0.99	1.03	36.7	35.8	0.98

PVD = plasma volume directly determined with T - 1824.   PVP = TVP - RVP.

RVP = red cell volume directly determined with P - 32.   RVD = TVD - PVD.

$$TBV = PVD + RVP. \quad TVD = PVD \times \frac{100}{1 - PHC}. \quad TVP = RVP \times \frac{100}{PHC}.$$

$$PHC = \text{observed hematocrit} \times 0.915. \quad BH = \frac{RVP}{TBV}.$$

an average ratio of 1.03. Only *case 16* shows a discrepancy greater than 15 per cent and 23 cases show an agreement to within 10 per cent.

Table 2 illustrates the results of a series of four determinations in a patient suffering from a severe infectious hepatitis whose metabolism was being studied in connection with various dietary procedures. Again the agreement in values over the 17-day period is excellent and within the errors of the methods.

As previously indicated, a crucial point in the present study was the determination of the magnitude of the discrepancy introduced into the blood volume determination because of unequal distribution of red blood cells in the vascular system. This can be estimated by comparing the average body hematocrit with the observed

TABLE 2.   PATIENT 15 WT. 68.2 KG.; SEVERE INFECTIOUS HEPATITIS

DATE 1948	PLASMA VOLUME, ML.		RED CELL VOLUME, ML.			TOTAL BLOOD VOLUME, ML.					HCT P.C.		
	PVD	PVP	RVP	RVD	$\frac{RVP}{RVD}$	TBV	TBD	TBP	$\frac{TBV}{TVD}$	$\frac{TBV}{TVP}$	PHC	BH	$\frac{BH}{PHC}$
April 27........	3900	4180	1810	1700	1.07	5710	5600	5990	1.02	0.95	30.2	31.7	1.04
May 3..........	4560	4470	2240	2290	0.98	6800	6850	6710	0.99	1.01	33.4	33.0	0.99
May 8..........	3830	3720	1980	2040	0.97	5810	5870	5710	0.99	1.02	34.8	34.0	0.99
May 14........	4100	4455	2145	1970	1.08	6245	6070	6600	1.03	0.94	32.5	34.4	1.06

TABLE 3.   PATIENT D.   COMPARISON OF SAMPLES FROM LARGE VESSELS

SOURCES OF BLOOD	TIME, MIN.	HEMATOCRIT P.C.		VOLUME P-32, ML.			VOLUME T-1824, ML.		
		Per.	Body	R. C.	Plasma	Total	R. C.	Plasma	Total
Antecubital V........	0	30.2							
Left femoral A.......	10	30.2	30.0	1430	3300	4730	1470	3370	4850
Hepatic V...........	12	30.2	30.0	1670	3855	5525	1700	3900	5600
Superior V.C........	30	30.2	29.5	1610	3740	5350	1660	3840	5500
Left subclavian......	35	30.2	32.7	1610	3715	5325	1440	3300	4740

(corrected) venous hematocrit. Such a comparison appears in table 1 and indicates a remarkable close agreement between the two values. Thus the average ratio for the entire series is 0.98 and again in only two instances are the differences greater than 10 per cent. Half of the cases show differences of 4 per cent or less and can certainly be considered as being identical.

Corroborative observations upon the identity of the corrected venous hematocrit and the body hematocrit have been obtained from several sources. In experiments with venous catheterization and simultaneous P-32 and T-1824 measurements, the corrected hematocrit from femoral artery, hepatic vein, caval and auricular blood has been in essential agreement with the peripheral venous hematocrit and the body hematocrit. Typical findings are presented in table 3. Estimation of total protein and hemoglobin concentrations in a number of experiments further confirms the identity of samples taken from these sites. Observations in one patient at the

time of splenectomy for familial jaundice have permitted comparisons with blood from the splenic artery and vein as well. Blood samples drawn from one of the antecubital veins, the splenic artery and the splenic vein gave hematocrit readings of 34.5, 34.7 and 34.5 per cent respectively. Red blood cell volumes calculated from these same samples gave values of 2000, 2178 and 2000 cc. respectively.

DISCUSSION

It must be apparent that correction of the value obtained for the peripheral venous hematocrit is the cardinal feature of this attempt to resolve the discrepancies between values obtained for red blood cell mass by direct and plasma-dye-hematocrit methods of estimation. Regardless of variation in centrifugation technics, it is to be expected that some plasma will be trapped in the sedimented red cells. It has seemed more important to us to adopt a standard centrifugation technic which permits application of a constant correction factor.

The uncorrected hematocrit gives a falsely high percentage of red blood cells which may be directly reflected in sense and magnitude in the calculation of red cell volumes from plasma-dye-hematocrit values. Similarly, any method of whole blood volume estimation which depends upon the peripheral venous hematocrit and a single direct measurement of either cells or plasma is open to error from this source.

Mathematical analysis of the predictable error indicates that failure to correct the venous hematocrit when a value of 40 per cent is obtained in the plasma-dye-hematocrit method would estimate a red cell mass 14 per cent in excess. Calculation of the whole blood volume would be too large by 5.3 per cent. In measurements of red cell volume by radioactive iron, the venous hematocrit is not used. However, estimation of whole blood volume by radioactive iron demands reference to the venous hematocrit and yields a value too small by 8.5 per cent.

Failure to correct for the error of the hematocrit is unquestionably responsible for many of the discordant results reported in the literature. Thus, if one analyzes the first five cases of comparative values for the plasma-dye-hematocrit and radioactive iron measurements as given by Gibson and his co-workers (1) the dye method yields red cell volumes 15 per cent higher than the iron method. Inasmuch as there was no correction of the hematocrit a discrepancy of 14.8 per cent was predictable. The average difference in whole blood volumes was 16.7 per cent and the average hematocrit was 42.6. Furthermore, applications of the correction factor of 8.5 per cent to the average value of 42.44 per cent for the venous hematocrit in the 40 normal males yields a value of 38.89 per cent. The average body hematocrit was 38.3 per cent. Correction of the average value for 40 dogs yields a value of 41.8 per cent for the venous hematocrit. The average body hematocrit was 41.5 per cent. In the same manner, correction of the peripheral venous hematocrit values in the 28 cases given by Meneely *et al.* (2) yields an average of 35.96 per cent as compared to their average body hematocrit value of 34.9 per cent. Reeve (8) has also discussed this point in a recent review.

On the other hand, those investigators who have used corrected hematocrits

have reported reasonably good agreement between the values determined from the red cell and plasma volumes respectively.   Thus Root, *et al.* (3), who used a factor of 0.96 to correct the hematocrit values, reported that in 13 of 14 measurements on resting and working men and in 23 of 29 animal experiments the blood volumes determined by the CO method agreed to within ±10 per cent with those obtained by the plasma-dye-hematocrit method.   Likewise, in all of their 14 human experiments and 26 of the 29 animal experiments, the ratio of body to peripheral venous hematocrit was between 0.90 and 1.08.

While the correction of the peripheral venous hematocrit serves to reconcile many of the discrepancies reported in the literature with respect to the plasma-dye-hematocrit and the various red cell volume methods, it may not be adequate to explain differences under conditions where there are shifts of plasma or blood from one area to another.   Since there is no proof that any of the available methods measure absolute blood volume, variations in vasomotor tone and blood flow may significantly influence the hematocrit ratios.   Trapping and loss of plasma from the active circulation might be expected to occur after transfusion, hemorrhage and shock which would make for discrepancies in the peripheral and body hematocrits which could not be reconciled by merely correcting the peripheral hematocrit.

The results of the present study do not disprove the claims that the cell to plasma ratio is less in the capillaries than in the large vessels.   They merely indicate that the amount of blood present in the small vessels is not large enough for the unequal distribution of red cells in these vessels to affect greatly the estimation of the blood volume as calculated from the dye and hematocrit reading under the conditions which we have studied.   Root *et al.* (3) arrived at similar conclusions from their comparison of the dye and the CO methods.

Routine correction for trapping in the small vessels would seem to be unnecessary and would introduce a definite error in the calculation of the blood volume. Our experience indicates that the plasma-dye method and the radioactive phosphorus methods measure circulating plasma and red cell volumes respectively with average discrepancies of less than 5 per cent and that whole blood volume can be calculated with the same accuracy from either determination if the corrected hematocrit value is used.   The random distribution of the data suggests that when large discrepancies are present, they may be due to errors in technic rather than to a fundamental and systematic error.

SUMMARY

Concomitant measurements of red cell mass and plasma volume have been made with the P-32 technic and the T-1824 method respectively in 10 'normal' and 35 hospitalized individuals.   A standard correction factor of 0.915 was used to correct the hematocrit values for trapped plasma.   Total blood volumes were calculated from the red cell volume and hematocrit and from the plasma volume and hematocrit respectively.   These values were compared with the total blood volume as calculated from the sum of the actually determined red cell and plasma volumes and showed satisfactory agreement.   Comparison of the peripheral and body hematocrits

also showed good agreement.   The data suggest that the whole blood volume can be measured with an average discrepancy of less than 5 per cent by the plasma-dye-hematocrit method providing the corrected hematocrit value is used.

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EFFECT OF THE ADMINISTRATION OF ADRENALIN ON THE CIRCULATING RED CELL VOLUME[1]

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THERE is now considerable evidence that in man there are no reserves of blood or blood cells which are subject to emergency mobilization (1–4). In spite of these observations, there has been considerable reluctance to abandon the earlier concept of splenic reserve of red blood cells as set forth by Barcroft in his original work on animals (5). Textbooks in physiology still discuss this emergency function of the spleen (6) and the concept is emphasized frequently in clinical teaching. As a result of increasing experience with blood volume technics, particularly with the tagged-red cell volume method described in the preceding paper (7), it became of interest to repeat certain earlier studies and collect simultaneous determinations of pulse rate, plasma volume, red cell mass, whole blood volume, plasma protein concentration, peripheral hematocrit, hemoglobin concentrations and body hematocrit after the subcutaneous injection of 1 mg. of adrenalin. It seemed especially pertinent to a final decision about the existence of reserves of red cells to compare the peripheral venous hematocrit with the body hematocrit before and after the adrenalin response. The term 'body hematocrit' is used to express the ratio of plasma volume to red cell mass as measured directly.

We have studied the changes induced by the subcutaneous injection of 1 mg. of adrenalin in 5 subjects. Three of these were healthy adults (2 males and 1 female) and 2 (1 male and 1 female) were hospitalized patients suffering from rheumatoid arthritis and hemolytic anemia respectively. One normal male, who expected an injection of adrenalin, was given an injection of 1 cc. of normal saline. All observations were made under basal conditions.

Simultaneous plasma and red cell volumes were determined with T-1824 (8) and P-32 (7) respectively and the total blood volume and body hematocrits calculated from these values. The peripheral (*in vitro*) hematocrit was determined by centrifuging blood samples for 30 minutes at 3000 r.p.m. in Wintrobe tubes. The distance from the axis of the centrifuge to the bottom of the tube was 20 cm. Hemoglobin was estimated as oxyhemoglobin and the falling drop method, checked by Kjehldahl determinations, was used for protein determinations. After control levels had been established, 1 mg. of adrenalin was injected subcutaneously and blood samples were obtained at approximately five-minute intervals for 30 minutes.

.The results are given in figure 1. A satisfactory 'adrenalin response' was obtained in every case (including the patient who received the injection of saline) as
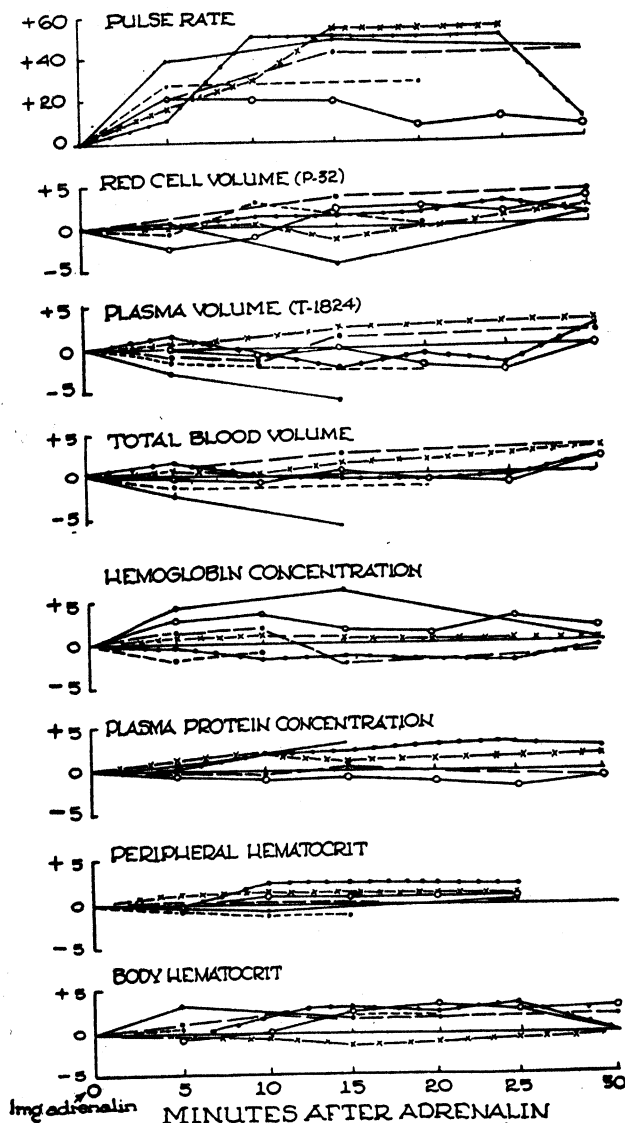
Fig. 1. EFFECTS OF SUBCUTANEOUS ADMINISTRATION of 1 mg. adrenalin. The values plotted are the percent changes from the average control (pre-injection) levels ·⊥⊥⊥⊥⊥ = T.B.C. arthritis; ————— = hemolytic anemia; - - - - - = normal-1 mg. saline injected instead of adrenalin; —×—×—×— = normal male; — — — — — = normal male; —O— = normal female.

attested by the rise in pulse rate and the subjective reactions of the subjects. The red cell, plasma and total blood volumes and the body hematocrit showed minor

changes which are within the error of the methods. The protein concentration and peripheral hematocrit showed a tendency to rise, but the hemoglobin concentration showed no definite trend. None of the changes, except for those in the pulse rate, were significantly greater than the changes observed in these functions during the control period before the administration of adrenalin.

Our results thus confirm and extend those of previous workers who failed to find any evidences of mobilization of red blood cells after the injection of adrenalin. The relatively consistent finding of increased peripheral hematocrit and protein concentration has suggested that the injection of adrenalin results in hemoconcentration. Kaltreider *et al.* (2) measured the plasma volume after adrenalin with T-1824 and reported a slight decrease in plasma volume in 8 of their 10 subjects. These changes were less than 8 per cent in all except one case. Our results fail to provide evidence for hemoconcentration, since, as mentioned above, the changes in plasma volume are random and within the errors of the method.

Ross and Chapin (3) have suggested that the increase in peripheral hematocrit is due to a redistribution of circulating cells and plasma within the vascular system. Such redistribution should manifest itself as a difference in the peripheral and body hematocrit (9). If redistribution does occur, the magnitude is such that it cannot be demonstrated because of the relative insensitivity of the methods in our experiments.

SUMMARY

The subcutaneous injection of adrenalin in an amount sufficient to evoke a good clinical response does not result in any uniform or significant changes in the plasma or red cell volumes as directly measured by the T-1824 and P-32 technics. If sympathetic stimulation or adrenalin influence these functions, the effect must be very slight and of no real significance as an emergency response.

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EFFECT OF STEROIDS ON THE BODY WEIGHT, TEMPORAL MUSCLE AND ORGANS OF THE GUINEA PIG[1, 2]

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THE preliminary report by Papanicalaou and Falk (1), that testosterone propionate increases the muscle mass of the female and the castrated male guinea pig, provided direct evidence that the protein-anabolic activity of androgens (2) was not directed entirely to the regeneration and maintenance of the accessory sex organs and other internal organs. It seemed advisable, therefore, to confirm (3) the myotrophic property of this androgen and to determine whether there might be steroids with a greater myotrophic than androgenic effect comparable to the renotrophic-androgenic effect observed in castrated mice (4–6).

METHODS

Animals. Immature, male, short-haired English albino guinea pigs were purchased from the Rockland farms. They were placed in individual metal screen cages (Norwich), fed *ad libitum* the Rockland guinea pig diet fortified with vitamin C and in addition a 12.5 mg. tablet of the vitamin was given by mouth each week. When the animals attained a body weight of approximately 250 gm., they were castrated in groups of 10 to 20 and treatment was begun 35 days later. At least one normal animal was included in the first five groups.

All of the steroids were studied for a 30-day period. In addition testosterone propionate as pellets was studied for 90 days, and for 14 days by injection at a dosage of 12.5 mg/day. Finally, one group of guinea pigs was castrated at approximately 600-gm. body weight, immediately implanted with testosterone pellets and compared with their normal controls 30 days later. Body weights were determined at approximately five-day intervals and on the day before and at autopsy.

Steroids.[4] The pellets of the various steroids were prepared as previously described (4). They weighed approximately 14 mg. each and were implanted subcutaneously by means of a trocar. The number of pellets implanted was determined on the basis of previous studies (3) and a series of preliminary experiments (7). The testosterone propionate[4] used for injections was provided in sesame oil at 25 mg/ml.

Autopsy. The guinea pigs were fasted 24 hours prior to autopsy when they were anesthetized by an intraperitoneal injection of dial-urethane[4] (usually 1.5 ml/kg. body wt.) and bled to death by cutting the blood vessels of the neck. The organs and temporal muscles were removed and weighed on a suitable Roller-Smith torsion balance.

A uniform portion of the ocular muscle was included with the temporal muscles because of its intimate attachment. The moisture content was determined by drying the muscle in an electric oven at 95 to 105°C. The non-protein nitrogen was determined on the trichloracetic acid filtrate of fresh muscle. Total nitrogen was determined on aliquots of the acid hydrolysate of the dried muscles. The nitrogen determinations were carried out by the micro-Kjeldahl technic.

RESULTS

Thirty-day Experiments

Body weight. The castrated guinea pigs did not gain as much weight as the normal animals (table 1). Implantation of pellets of the various steroids restored the body weight toward normal, but when a maximum response was attained further increase in dose either had no further effect or was less effective.

TABLE 1. EFFECT OF STEROIDS ON BODY WEIGHT, KIDNEYS AND ADRENALS OF CASTRATED GUINEA PIGS[1]

	NO. OF G. PIGS	STEROID		BODY WEIGHT		KIDNEYS	ADRENALS
		Pellets	Absorbed	Initial	Change		
		No.	*mg/30 days*	*gm.*	*gm., %*	*gm., %*	*gm., %*
Castrate	15			460	(140)	(4.100)	(0.244)
Normals	6			507	47	−2	+13
Androstanol-17α, one-3	5	1	1.9	467	41		
	6	3	5.0	464	38	10	−22
	5	6	9.1	449	30	2	−11
17-Methylandrostanol- 17α, one-3	5	1	1.5	455	−13	−6	
	5	3	4.4	469	39	6	−14
Testosterone propionate	5	1	4.0	457	5		
	6	2	7.5	464	52	2	0
Testosterone	5	1	7.4	446	51	−1	7
	5	3	18.9	458	45	2	13
	5	5	32.2	448	35	16	
17-Methyltestosterone	5	1	6.8	478	36	5	3
	5	3	17.7	446	44	8	11
	5	5	28.3	451	39	6	
Androstanediol-3α, 17α	5	3	5.8	455	19	−2	5
	4	12	14.5	488	43		
17-Methylandrostanediol- 3α, 17α	5	1	1.9	466	−13	−9	
	5	6	9.5	454	33	2	−16

[1] Average values of the control guinea pigs are given in parentheses. The differences are from these values.

Kidneys and adrenals. There was no significant change in the size of these organs either as a result of castration or the implantation of the various steroids as pellets (table 1).

Myotrophic effects. Castration decreased the weight of the temporal muscles to less than one-third that of the normal animals. All of the steroids increased the weight of the temporal muscle of the castrated guinea pig, but in no instance was there a restoration to the normal size (fig. 1). Indeed the greatest increase was only about one-half that of the normal animals.

· The myotrophic property was different for each steroid. Androstanol-17α, one-3 proved to be the most potent compound. Testosterone and testosterone propionate had the same effect and androstanediol-3α,17α was very ineffective at a low dose level but on increasing the dose became very effective. The 17-methyl derivatives especially methyl testosterone were less effective than their parent steroids.
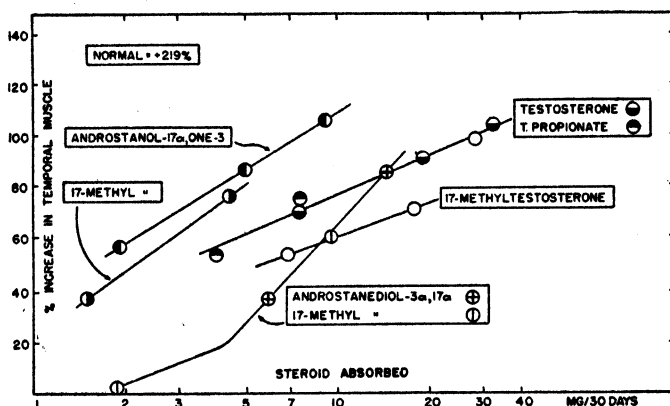
Fig. 1. Myotrophic property of steroids
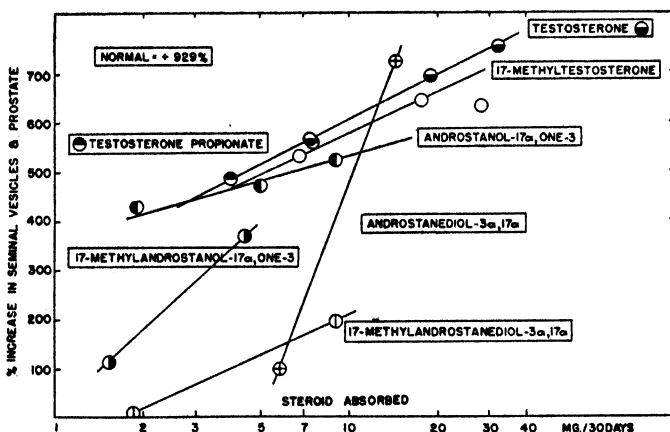
Fig. 2. Androgenic property of steroids

Androgenic effects. There was a definite stimulation of the seminal vesicles and prostates of the castrated guinea pigs by all of the steroids (fig. 2). At the higher dose levels they produced accessory sex organs only slightly smaller than those of the normal guinea pigs. The order of androgenic potency of the steroids was different from their myotrophic effects. Testosterone and testosterone propionate were the most potent. The 17-methylated compounds were again less effective than their parent steroids.

Myotrophic: androgenic ratio. The relative myotrophic-androgenic property

of the various steroids is illustrated by dividing the increase in temporal muscle mass by that of the seminal vesicles and prostates (fig. 3). The normal guinea pigs have a ratio of 0.311 and all of the steroids, except the diols and 17-methylandrostanol-17α,one-3, have lower ratios. As the dose of steroid exhibiting the high ratios is increased, there is a much greater increase in androgenic than myotrophic effect resulting in a rapid decrease of the ratio. The decrease, however, is not sufficient to lower the ratio below that of the other steroids at comparable dose levels. The steroids with the low ratios, however, progress towards the normal with further ncrease in dose.

Fig. 3. Effect of dose on the myotrophic-androgenic ratio.
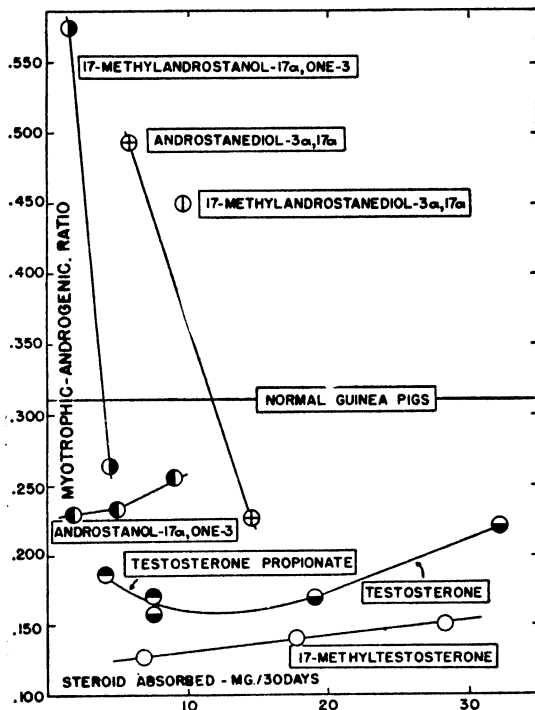
Ninety-day experiments. The extension of the time of stimulation by testosterone propionate pellets to 90 days indicates that the seminal vesicles and prostates have been nearly returned to normal (fig. 4). Indeed the nature of the growth curve suggests that a further 30 days of treatment would have completely restored the size of these organs. The temporal muscles, on the other hand, are still very much smaller than those of the normal guinea pig and it would require an indefinite period of stimulation at the dose employed to accomplish complete restoration. The myotrophic and androgenic ratio was 0.323 for the normal guinea pigs and 0.167 for the androgen treated animals.

Injection of a large dose of testosterone propionate. The subcutaneous injection of 12.5 mg/day of testosterone propionate produced effects similar to those seen

by the subcutaneous implantation of pellets (table 2). This large dose of androgen was unable to restore the temporal muscle or the accessory sex organs to normal.
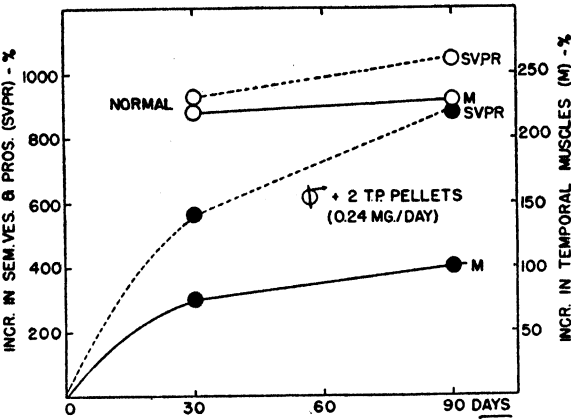

Fig. 4

TABLE 2. EFFECT OF INJECTION OF A LARGE DOSE OF TESTOSTERONE PROPIONATE (T.P.) ON CASTRATED MALE GUINEA PIGS

	NO. OF G. PIGS	BODY WT.		TEMPORAL MUSCLE (M)	SEM. VES. + PROS. (A)	M/A	KIDNEYS
		Initial	Change				
		gm.	gm., %	gm., %	gm., %	mg/mg.	gm., %
Control.................	5	575	(56)	(0.640)	(0.384)		4.280
T.P. 12.5 mg/day.........	5	579	+39	+50	+462	0.180	0

TABLE 3. MAINTENANCE OF THE TEMPORAL MUSCLE OF CASTRATED GUINEA PIGS BY TESTOSTERONE

	NO. OF PIGS	PELLETS	AB-SORBED	BODY WEIGHT		TEMPORAL MUSCLE	SEM. VES. AND PROS.	KIDNEY
				Initial	Gain			
			mg/30 days	gm.	gm., %	gm., %	gm., %	gm., %
Normals.............	4			602	(120)	(1.900)	(3.510)	(4.800)
Testosterone[1].........	4	3	21.3	604	−7	0	+43	0
Testosterone..........	2	5	33.3	580	+50	−5	+74	+8

[1] Pellets implanted at time of castration.

Indeed the myotrophic and androgenic effects were equivalent to that observed after the absorption from a pellet of 0.1 mg/day for 30 days (table 1).

Maintenance of temporal muscles and accessory sex organs. Since it was so difficult in the preceding experiments to restore the size of the temporal muscle to normal size, it seemed important to determine whether these could be maintained

at the normal level.   Therefore, a group of guinea pigs were castrated 35 days after the usual time and at the same time were implanted with pellets of testosterone.   At autopsy 30 days later (table 3) the temporal muscles had been perfectly maintained at a dose of 0.7 mg/day.   At the higher dose level of 1.1 mg/day there was no

TABLE 4.   COMPOSITION OF THE TEMPORAL MUSCLES OF GUINEA PIGS TREATED
WITH VARIOUS ANDROGENS

	NO. OF G. PIGS	STEROID ABSORBED	TEMPORAL MUSCLE	WATER	PROTEIN (Nx6.25)	REST
		mg/30 days	*mg.*	%	%	%
Normal.............................	6		1746	77.5	19.05	3.45
Control.............................	6		547	76.1	17.95	5.95
Testosterone.........................	5	7.4	931	76.5	18.95	4.55
Testosterone.........................	5	18.9	1044	76.6	19.50	3.90
Testosterone propionate...............	6	7.5	958	76.3	18.85	4.85
17-Methyltestosterone................	5	6.8	836	75.8	19.00	5.20
17-Methyltestosterone................	5	17.7	935	76.3	19.00	4.70
Androstanol-17α, one-3...............	6	5.0	1017	76.6	18.90	4.50
17-Methylandrostanol-17α,one-3.......	5	4.4	963	76.2	18.45	5.35
Androstanediol-3α,17α...............	5	5.8	757	76.2	18.27	5.53
17 Methylandrostanediol-3α,17α.......	5	9.5	877	76.1	19.15	4.75

T.P. injected at 12.5 mg/day for 14 days

| Control............................ | 4 | | 640 | 75.? | 18.20 | 6.10 |
| Testosterone propionate.............. | 4 | | 960 | 75.7 | 18.57 | 5.73 |

Fig. 5
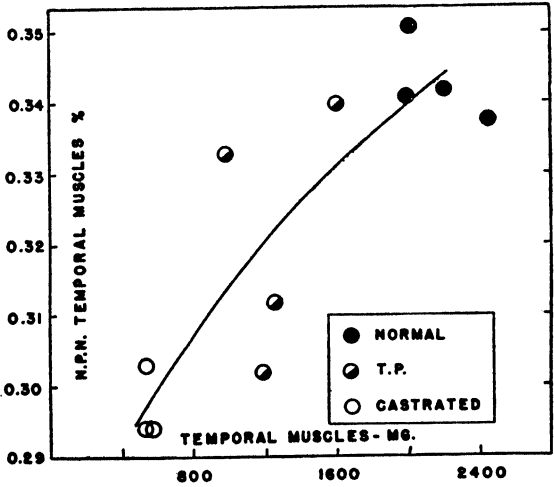
greater stimulation.   The accessory sex organs, on the other hand, were greatly increased at the lower dose and further stimulated at the higher dose.   The kidneys showed no significant changes.   The increase in body weight was unaffected at the lower dose but definitely enhanced at the higher dose.

. *Composition of the temporal muscles.* There is a slight decrease in both percentage of total water and total protein (nitrogen × 6.25) of the temporal muscles as a result of castration. The protein but not the water content is restored toward the normal level by the steroids (table 4).

In one series of experiments the nitrogen not precipitable by trichloracetic acid (N.P.N.) was determined. Castration decreased the percentage of this mixture of nitrogen containing substances but testosterone propionate restored them towards normal (fig. 5).

DISCUSSION

It is noteworthy that as in the case of the renotrophic and androgenic effects in mice (4–6) none of the steroids was able to simulate the normal myotrophic and androgenic status of the normal guinea pig. It seems, therefore, that neither testosterone, in spite of its isolation from bulls' (8) and horses' (9) testes, nor any other of the steroids are 'the hormone' of the guinea pig or mouse testes. Therefore, if a single substance is primarily responsible for the development and maintenance of
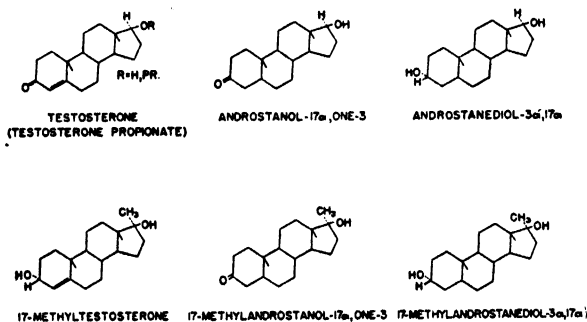

TESTOSTERONE
(TESTOSTERONE PROPIONATE)

ANDROSTANOL-17α,ONE-3

ANDROSTANEDIOL-3α,17α

17-METHYLTESTOSTERONE

17-METHYLANDROSTANOL-17α,ONE-3

17-METHYLANDROSTANEDIOL-3α,17α)

Fig. 6. STERIC CONFIGURATION of the 17-hydroxyl group has been indicated according to the recent revision (14); the designations of α and β, however, have been maintained since these were introduced (15) in anticipation of such revisions.

these tissues, it has not been recognized as yet. On the other hand, a group of substances in proper combination may be necessary or the available methods of study do not provide an adequate simulation of normal conditions.

The high myotrophic-androgenic ratios produced by the diols recalls the very similar renotrophic-androgenic ratios produced in castrated mice by these steroids (4–6). The different myotrophic-androgenic ratios of the different steroids and their increase or decrease with dose, depending on the particular steroid, tempts speculation as to whether these changes may not be a reflection of the nature of the metabolites formed by the body from the administered steroids. Thus, the diols especially at lower doses would be converted by the body primarily to metabolites which are myotrophic while the testosterone compounds are metabolized primarily to androgenic steroids.

The observation that androstanol-17α,one-3 is the most potent myotrophic steroid serves to illustrate again that the various properties of the steroids do not parallel each other. Furthermore, it illustrates that a slight alteration in chemical structure (fig. 6) can change a physiological property of a steroid.

The analyses of the muscles indicate that the growth is similar to that present

in normal animals and provides direct support for the many calculations made on the basis of changes in the urinary excretion of constituents necessary for the formation of protoplasm after androgenic stimulation (2, 10).

The much greater efficacy of a steroid when administered by pellet as compared with injection is well illustrated again. Pellets of testosterone propionate were roughly one hundred times as effective as injections.

The failure of castration or any of the steroids to affect the size of the kidney of the guinea pigs is in marked contrast to that observed in the mouse (4–6), but in agreement with the observations in the hamster (11) and the rabbit (12). The hamster, however, is different from the guinea pig (also most other mammals), with respect to body size. The female of this species is larger than the male and has larger skeletal muscles. Castration of the male produces the female type (13).

The ability of testosterone to maintain the temporal muscles at exactly normal size indicates that the demands for restorative processes are much greater than those for maintenance. A similar dose of testosterone was able to restore the atrophied muscle of the castrated guinea pig only to approximately 50 per cent of normal.

SUMMARY

Male guinea pigs were castrated at 250 gm. body weight and 35 days later were implanted subcutaneously with pellets of various steroids. After 30 days the animals were autopsied. Castration decreased the temporal muscles to less than one-third that of the normals. The steroids increased the muscles in the following descending order: androstanol-17α,one-3; 17-methylandrostanol-17α,one-3; testosterone and testosterone propionate; 17-methyltestosterone; androstanediol-3α,17α; and 17-methylandrostanediol-3α,17α. The dose response was logarithmic for all of the compounds except androstanediol-3α,17α, which became as effective as testosterone at higher dose levels. The myotrophic activities did not parallel the androgenic properties of these steroids. Therefore, the myotrophic-androgenic ratio (increase of muscle weight divided by the increase in accessory sex organs) for the various steroids decreased as follows: androstanediol-3α,17α; 17-methylandrostanediol-3α,17α; 17-methylandrostanol-17α,one-3; androstanol-17α,one-3; testosterone and testosterone propionate; and 17-methyltestosterone.

The temporal muscles were never restored to more than 50 per cent of the normal, but the accessory sex organs were restored. If pellets of testosterone were implanted at time of castration, the muscles were maintained at exactly the same weight as those of normal controls, but the seminal vesicles and prostates were greatly increased. The normal condition was not simulated by any of the steroids. Analyses of the muscles indicated that normal growth occurred under steroid stimulation.

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EFFECT OF CASTRATION AND STEROIDS ON THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE GUINEA PIG[1,2]

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CERTAIN androgens produce remarkable increases in the arginase activities of the kidney of the mouse (1-3), moderate increases in the kidney of the rat (4) and a decrease in that of the hamster (5). These steroids decrease the 'alkaline' phosphatase of the kidney of the mouse at 'high' doses (6), but produce a moderate increase at 'low' doses (7) and similar increases in that of the rat (4) and hamster (5). The 'acid' phosphatase of the kidney is increased in the mouse (6), but not changed in the rat (4) or the hamster (5). These enzymes of the liver are not changed by administration of androgens in any of the above species.

The above studies now have been extended to another species, the guinea pig.

PROCEDURE

The guinea pigs were those used in the preceding report (8) At autopsy approximately one gm. of the left section of the median lobe of the liver and one half of one kidney were weighed, placed in 5 ml. of cold redistilled water, homogenized and sufficient water added to make a concentration of 20 ml. of water/gm. of tissue. The enzymes were determined as previously described (1, 2, 4, 6).

RESULTS

Kidney arginase. The normal guinea pigs showed a significantly greater arginase activity than their castrated controls in the 30-day experiments, but this difference disappeared on extension of the time to 90 days (table 1A).

None of the steroids produced a remarkable increase in the arginase of the kidney (table 1A); 17-methyltestosterone and 17-methylandrostanediol-3α,17α produced the greatest increases. The latter steroid, however, was more effective than the former at similar doses. Testosterone was completely ineffective at all dose levels while its propionate produced a small but not significant change at 30 days, which was not apparent at 90 days and was enhanced to a significant increase by the injection of the relatively large dose of 12.5 mg/day for 14 days (table 1B). 17-methylandrostanol-17α,one-3 at the higher dose and androstanol-17α,one-3 produced small increases in arginase activity. Androstanediol-3α,17α at a similar dose level was ineffective.

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Kidney 'alkaline' phosphatase. The kidney of the normal guinea pigs demonstrated a higher 'alkaline' phosphatase activity than that of their castrated controls. This difference, however, was not apparent in the older animals (table 1A). All of

TABLE 1. EFFECT OF CASTRATION AND VARIOUS STEROIDS (PELLETS) ON ARGINASE AND 'ALKALINE' PHOSPHATASE OF THE GUINEA PIG KIDNEY[1]

	NO. OF G. PIGS	TOTAL STEROID ABSORBED	KIDNEY WT.	ARGINASE[2]	'ALKALINE' PHOSPHATASE[2]
		mg/30 days	*gm.*	*(U/gm.) %*	*(U/gm.) %*
A. 30-Day Experiments					
Castrated controls	8		3.935	(31) (22–40)	(84) (63–112)
Normal controls	6		4.039	+58	+63
17-Methyltestosterone	5	28.3	4.344	+79	+43
	5	17.7	4.405	+41	+24
	5	6.8	4.319	+40	+39
Testosterone	5	32.2	4.451	−15	+24
	5	18.9	4.513	−3	+42
	5	7.4	4.204	+5	+39
Testosterone propionate	6	7.5	4.296	+27	+36
17-Methylandrostanol-17α, one-3	5	4.4	4.344	+36	+40
	5	1.8	3.998	+4	+50
Androstanol-17α, one-3	5	9.1	4.193	+50	+71
	6	5.0	4.499	+33	+41
17-Methylandrostanediol-3α, 17α	5	9.5	4.168	+73	+29
	4	1.9	3.727	−11	+35
Androstanediol-3α, 17α	5	5.8	4.023	+22	+19
B. 90-Day Experiments					
Castrated controls	4		5.074	(31) (26–36)	(97) (85–104)
Normal controls	4		4.990	−16	+29
Testosterone propionate	4	20.5	5.117	+10	+28

[1] There was no significant change in the 'acid' phosphatase of the kidney nor in any of the enzymes of the liver.

[2] Average and range of values for the controls are given in parentheses. The % values are differences from the average values of the controls.

the steroids restored in varying degrees the decrease in activity of the phosphatase toward normal. The increases, however, were not related to the dose or the chemical nature of the steroid.

Kidney 'acid' phosphatase. The changes in this enzyme were generally less than 5 per cent and never more than 10 per cent. The changes were not significant and are not presented.

Liver enzymes. There were no significant changes in the activities of the enzymes of the liver. Most of the differences were below 5 per cent and in a few instances as much as 18 per cent.

DISCUSSION

The failure of castration and the steroids to influence any of the liver enzymes is the same as the observations in the mouse (1-3, 6, 7), rat (4) and hamster (5). Thus, in the guinea pig as in the other species the protein anabolic effect of these steroids (9) does not require a change in the activities of these enzymes.

The ability of 17-methyltestosterone to increase the arginase activity of the kidney is apparently due to the 17-methyl group, for testosterone at comparable

TABLE 2. EFFECT OF INJECTION OF A 'LARGE DOSE' OF TESTOSTERONE PROPIONATE (T.P.)
ON KIDNEY ENZYMES OF CASTRATED MALE GUINEA PIGS[1]

	NO. OF G. PIGS	DOSE		KIDNEY WT.	ARGINASE	PHOSPHATASES	
						'ALKALINE'	'ACID'
		mg/day	*days*	*gm.*	*U/gm.*	*U/gm.*	*U/gm.*
Controls	5			4277	29 (19-33)	76 (62-85)	21 (20-23)
T.P.	5	12.5	14	4360	40 (36-45)	79 (70-83)	21 (20-22)
Change %					+38	+4	0

[1] There was no significant change in the enzymes of the liver.

dose levels was completely ineffective. The increase, however, is much smaller than that observed in the mouse (1-3) and even lower than in the rat (4). Furthermore, an attempt to obtain a great increase in arginase activity by the injection of the relatively large dose of 12.5 mg/day of testosterone propionate was unsuccessful. The increase produced was very small and in no manner comparable to that in the mouse (2).

The ability of the steroids to increase the 'alkaline' phosphatase of the kidney is comparable to that noted in the mouse at subnormal doses (7) and in the rat (4) and hamster (5) regardless of dose.

SUMMARY

Male guinea pigs were castrated at about 250 gm. body weight. Thirty-five days later they were implanted subcutaneously with pellets of the following steroids: 17-methyltestosterone; testosterone; testosterone propionate; 17-methylandrostanol-17α,one-3; androstanol-17α,one-3; 17-methylandrostanediol-3α,17α; and androstanediol-3α,17α. The dose of steroid was varied by the number of pellets implanted. Castration produced a decrease in the arginase activities of the kidney after 60 days, but not after 120 days. None of the steroids produced any remark-

able ·changes.   The greatest increase, 79 per cent, was produced by 17-methyltestos-terone while testosterone was completely ineffective.   The administration of a relatively large dose, 12.5 mg/day, of testosterone propionate for 14 days produced only a 38 per cent increase.   Castration produced a decrease in the 'alkaline' phos-phatase of the kidney, which was restored toward normal by the various steroids. None of the enzymes of the liver or the 'acid' phosphatase of the kidney were affected by castration or the steroids.

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EFFECT OF TESTOSTERONE PROPIONATE AND GROWTH HORMONE ON THE WEIGHTS AND COMPOSITION OF THE BODY AND ORGANS OF THE MOUSE[1, 2]

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THE steroid N-hormones, e.g. testosterone (1) and the growth hormone of the anterior pituitary (2-4), are very potent stimulators of protein anabolism. It seemed of importance, therefore, to make a comparison of the sites and nature of the growth stimulated by these two important hormones.

PROCEDURE

Animals. White male mice of the Swiss strain were castrated at 17- to 19-gm. body weight; treatment was begun 30 to 40 days later. They were maintained in a glass jar containing wood shavings in an air-conditioned room at 78 to 80°F. They were fed the Rockland rat diet and tap water *ad libitum.* Body weights were recorded three times per week beginning one week before treatment.

Hormones. The testosterone propionate[3] was implanted subcutaneousl. as a pellet of approximately 14 mg. (5). The growth hormone extract was prepared from beef pituitary. It contained "1.1 units/mg. in the 6-month old 'plateaued' female rat growth test"[4] and "in the hypophysectomized immature female rat,[5] 0.1 mg/day for 10 days produced an average increase of 14.1 gm. in body weight. It showed no corticotrophin activity but at a total dose of 2.5 mg. it repaired the interstitial cells of the ovary and the thyroid of the hypophysectomized rat".

The extract was supplied as a dry powder and was dissolved as follows: 100 mg. was placed in a 15-ml. centrifuge tube set in an ice water bath, 8 ml. of ice cold N/100 sodium hydroxide was added and the suspension vigorously stirred at intervals for 30 to 60 minutes. Then the mixture was titrated, to pH 9 (Universal indicator, Eastman Kodak Co.) with N/50 phosphoric acid. It was made to 10 ml. with distilled water, stirred and centrifuged. The supernatant solution was decanted into rubber-capped vials and kept at 0 to 5°C. A maximum of one week's supply was prepared at one time. It was injected at 0.1 ml. (1.1 u.) per day.

Autopsy and preparation of tissues. The food was removed 18 to 24 hours before the end of the experiment. The mice were killed by severing the spinal chord at the base of the skull and bleeding from the blood vessels of the neck. The organs were removed and weighed on a Roller-Smith torsion balance. The kidneys and livers were homogenized (6) and aliquots removed for enzyme (7) and nitrogen determinations. The gastro-intestinal tract was freed of fecal material and the carcass and contents were dried to constant weight at 80 to 90°C. in an electric oven attached to a water aspirator. The dried residue was dissolved with warming in 30 ml. of 50 per cent potas-

sium hydroxide and 30 ml. of redistilled alcohol and transfered to a 200-ml. volumetric flask. The bones were dissolved in 3 N hydrochloric acid. The mixture then was made to volume with distilled water.

The seminal vesicles and prostates and the remainder of the kidney and liver homogenates were pooled in separate groups of 3 to 5, dried to constant weight as above, dissolved in 10 ml. of 50 per cent potassium hydroxide and 10 ml. of redistilled alcohol and made to 50 ml.

Nitrogen determination. The micro-Kjeldahl procedure was used for all nitrogen determinations except that aliquots of the dissolved carcasses were digested by the macro procedure, made to a given volume from which samples were distilled by the micro procedure.

Total fat determination. A modified Leathes and Raper method (8, cf. 9) was used. An aliquot of the dissolved tissue was pipetted into a 250-ml. centrifuge bottle containing crushed ice, an equal volume of 6 N hydrochloric acid was added and the mixture extracted three times with 75 ml. of distilled petroleum ether (Skellysolve F). The pooled extract was washed once with 50 ml. of 40 per cent alcohol which was in turn extracted twice with fresh petroleum ether. The bottles were centrifuged after each extraction and the Skellysolve F removed by aspiration. The extracts were combined, concentrated to about 10 ml., transferred to a tared 50-ml. erlenmeyer flask, evaporated to dryness and placed in a vacuum desiccator containing 'dehydrite' for at least 24 hours before weighing.

RESULTS AND DISCUSSION

Body weight. The chosen doses of testosterone propionate and the growth hormone extract produced similar increases in the body weight except that the rate of increase was slightly different (fig. 1). The mice treated with the androgen showed an initially more rapid rate of increase, which decreased to less than that of the growth-hormone treated mice after about seven days of treatment. The growth curves of the 10- and 20-day treated mice were similar to the respective portions of the growth curves of the 34-day treated mice (fig. 1).

Organ weights. The change in body weight was due in part to changes in organ weights. Testosterone propionate produced the expected increase in the accessory sex organs. The growth hormone was ineffective when given alone or simultaneously with testosterone propionate (table 1). Testosterone propionate produced a progressive increase in the size of the kidneys with duration of treatment. The growth hormone produced a much smaller increase in this organ and no further increase was obtained on extending the period of injections. The simultaneous administration of the hormones produced a partial summation of their renotrophic effects.

There was a small increase (table 1) in the size of the liver in all of the mice except those treated with the androgen for 10 days. There was no evidence of summation when the two hormones were administered simultaneously. The androgens decreased the size of the thymus but the growth hormone was ineffective. There was no gross evidence of adrenal changes.

Composition of the organs. There was no major change in the percentage composition of the kidney or the liver (table 2) as a result of the various treatments. The seminal vesicles and prostates (table 3) had approximately the same percentage composition of water and protein in the various groups, but the fat content was decreased in the animals treated for 34 days. The organs of the control animals were too small (cf. table 1) to permit analyses.

Composition of the carcass. The water content of the carcass was progressively increased by both hormones. The increases, however, completely disappeared in the

10-day treated mice and become very small in the longer treated animals when the calculations are made on a fat-free basis (cf. 10, 11). The increases, nevertheless, are always greater after growth-hormone than androgen treatment and there is also a

Fig. 1. EFFECTS OF (TESTOSTERONE PROPIONATE (T. P.) and growth hormone (G. H.) on body wt. of castrated mice.
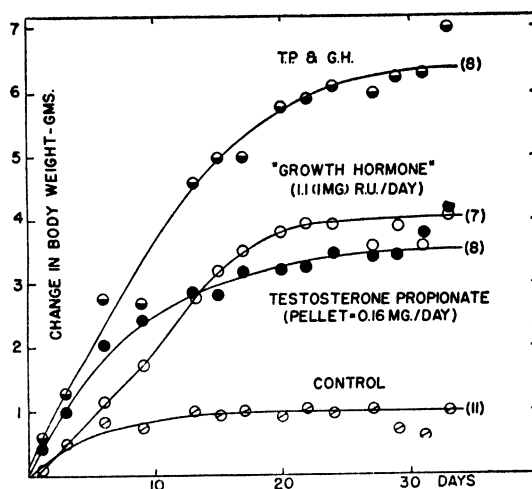
TABLE 1. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. F.) AND AN ANTERIOR PITUITARY GROWTH HORMONE (G. H.) ON THE ORGAN WEIGHTS OF CASTRATED MICE

	mg/day	days	NO. OF MICE	SEM. VES. + PROS. (mg), %[1]	KIDNEYS (mg), %[1]	LIVER (mg), %[1]	THYMUS (mg), %[1]
Controls			19	(10) (5–14)	(234) (193–260)	(920) (720–1010)	(47) (34–71)
T. P.	0.15	10	6	1600	57	4	−60
	0.16	20	9	2900	73	20	−95
	0.15	34	8	3500	81	15	−95
G. H.	1.0	10	9	30	37	29	+6
	1.0	20	9	10	30	35	+7
	1.0	34	7	30	24	11	−18
T. P. and G. H.	{0.16 {1.0	20	8	3000	93	31	−95
	{0.16 {1.0	34	8	4200	91	20	−95

[1] Percentage difference from the control values; the averages and range of values are given as milligrams in parentheses.

suggestion of partial summation when both hormones are administered. The nitrogen content was not remarkably altered. The quantity of fat was decreased by both hormones but the growth hormone was more effective than the androgen.

Composition of the increase in body weight. A summary of the total changes in all

of the organs and carcass is presented in figure 2. The increase in body weight over that of the controls occurred as a result of protein synthesis. Furthermore, when the two hormones were administered simultaneously there was a summation of their protein anabolic properties accompanied by a loss in body fat, which occurred entirely in the carcass (table 4). Indeed the organs, especially the seminal vesicles and prosstates, synthesized fat in proportion to their increases in weight (tables 1–3). The

TABLE 2. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND GROWTH HORMONE (G. H.) ON COMPOSITION OF THE KIDNEY AND LIVER OF THE CASTRATED MOUSE

	NO. OF MICE	KIDNEY			LIVER		
		Water	Nitrogen	Fat	Water	Nitrogen	Fat
		%	%	%	%	%	%
Controls.............	13	73.8	3.03	3.9	67.0	3.50	5.5
10-Day Experiments							
T. P.................	6	74.5	2.95	3.4	65.6	3.57	6.1
G. H.................	9	75.2	2.94	3.8	66.2	3.54	4.8
20-Day Experiments							
T. P.................	9	74.6	3.03	3.3	66.8	3.67	4.3
G. H.................	9	74.8	3.12	3.3	67.1	3.58	4.5
T. P. + G. H.........	8	74.8	3.02	3.2	67.1	3.70	4.3
34-Day Experiments							
T. P.................	8	74.2	2.89	3.7	65.4	3.94	4.6
G. H.................	7	74.8	2.96	3.8	68.8	3.63	3.8
T. P. + G. H.........	8	75.8	3.00	3.0	67.3	3.86	4.2

TABLE 3. EFFECT OF TESTOSTERONE PROPIONATE (T. P.) ALONE AND WITH GROWTH HORMONE (G. H.) ON COMPOSITION OF THE SEMINAL VESICLES AND PROSTATES OF CASTRATED MICE[1]

	10 DAYS			20 DAYS			34 DAYS		
	Water	Nitrogen	Fat	Water	Nitrogen	Fat	Water	Nitrogen	Fat
	%	%	%	%	%	%	%	%	%
T. P.................	73.8	3.10	3.9	71.7	3.60	3.4	73.5	3.41	2.0
T. P. + G. H.........				71.8	3.40	4.2	72.7	3.50	2.5

[1] The seminal vesicles and prostates of the control and growth-hormone-injected mice were too small (cf. table 1) to permit analyses.

amounts, however, are relatively insignificant when compared to the changes in the carcass.

In every instance the greatest synthesis of protein occurred in the carcass (table 5) and became greater with increase in duration of treatment. The amount, however, was less in the androgen- than in the growth-hormone-treated mice because of the greater synthesis of protein in the kidney and especially the seminal vesicles and prostates.

The effect of the growth hormone on the body and organ weights and their composition is in general agreement with previous studies in the mouse (9, 12) and

Fig. 2. COMPOSITION OF THE INCREASE IN BODY WT. of castrated mice treated with testosterone propionate (T. P.) and growth hormone (G. H.) over that of control animals.
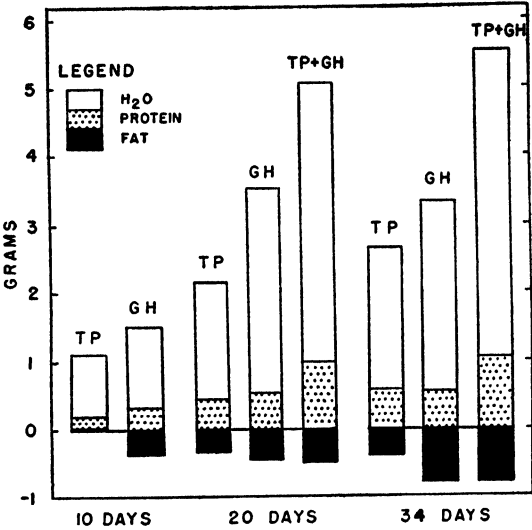
TABLE 4. EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND GROWTH HORMONE (G. H.) ON COMPOSITION OF THE CARCASS OF CASTRATED MICE

	NO. OF MICE	WATER	NITROGEN	FAT	FAT FREE BASIS	
					Water	Nitrogen
10-*Day Experiments*						
		%	%	%	%	%
Control	7	61.9	2.91	12.6	70.5	3.41
T. P.	6	62.3	2.88	10.9	69.5	3.23
G. H.	9	63.8	2.94	10.3	71.5	3.23
20-*Day Experiments*						
Control	7	62.1	2.91	12.6	70.5	3.41
T. P.	8	63.9	2.93	10.2	71.4	3.27
G. H.	9	65.3	2.82	9.2	72.1	3.11
T. P. + G. H.	8	66.5	2.95	8.4	72.4	3.22
34-*Day Experiments*						
Control	5	61.8	2.91	12.6	71.2	3.37
T. P.	8	64.4	3.04	9.8	72.0	3.37
G. H.	7	67.7	3.02	7.8	72.8	3.24
T. P. + G. H.	8	67.8	3.04	7.2	73.6	3.29

the rat (3, 13, 14). There are, however, contradictory reports concerning the effect of this hormone on the liver and kidney weights of the rat (cf. 15).

The chosen doses of the two hormones fortunately produced nearly identical increases in body weight. Thus a comparison of the results of the two types of treat-

ment are greatly facilitated. The effect of these two hormones are distinctly different. The growth hormone as expected had no androgenic effect in contrast to the testosterone propionate. The renotrophic properties also were decidedly different. The androgen not only produced larger kidneys but also progressively increased the size with extension of treatment, while the growth hormone produced only a small increase in kidney weight which was not increased further by extending the duration of treatment.

The simultaneous administration of the two hormones produced almost an exact summation of increase in body weight and a partial summation of their renotrophic effects. It is unlikely that these results are due to a further stimulation of the same intermediary metabolic processes, for doubling the dose of testosterone propionate does not produce a further increase in the body weight of the castrated mouse. There is, however, a slight increase in the size accompanied by a further increase in the arginase activity of the kidney (16). On the other hand, the growth hormone inhibits the increase in arginase activity of the kidney produced by testosterone propionate (7).

TABLE 5. SITES AND RELATIVE AMOUNT OF PROTEIN ANABOLISM INDUCED BY TESTOSTERONE PROPIONATE (T. P.) AND GROWTH HORMONE (G. H.) IN CASTRATED MICE

	10 DAYS		20 DAYS			34 DAYS		
	T. P.	G. H.	T. P.	G. H.	T. P. + G. H.	T. P.	G. H.	T. P. + G. H.
	%	%	%	%	%	%	%	%
Carcass............	69.2	76.7	65.3	80.4	79.4	75.5	92.3	83.0
Liver..............	2.8	19.5	10.6	16.8	9.3	8.7	6.2 .	5.5
Kidney............	11.3	4.5	7.1	2.8	4.3	4.7	1.5	3.4
Seminal vesicles and prostates.........	16.7	0.0	16.8	0.0	7.0	11.1	0.0	8.1

Values are percentages of the increase in body weight (see fig. 1).

The total composition of the increase in body weight is in general the same for the two hormones. There was an increase in protein synthesis and an increase in fat catabolism. The amount of fat catabolism, however, was greater and occurred sooner in the growth-hormone than in the androgen-treated mice. Furthermore, there was no apparent summation of this effect, while there was in protein synthesis when the two hormones were administered simultaneously. The increase in fat catabolism agrees with the decreases in R. Q. observed after administration of growth hormone (17) and androgens (1, 18) to dogs.

The site of greatest protein anabolism and all of the fat catabolism occurred in the 'carcass'. The kidney, liver and especially the seminal vesicles and prostates of the androgen treated mice were sites of not only protein but also fat synthesis. The protein synthesized in the carcass was similar to that in young growing animals (10) especially in the growth-hormone-treated mice. The composition of the kidney and liver was the same as that in the control animals.

The stimulation of protein synthesis in the carcass by the androgen presumably occurred in the skeletal muscles. These tissues of the castrated guinea pig are in-

creased in size by testosterone propionate (19) and related steroids (20). The skin also may have either participated or detracted from the changes noted since its hair growth and appearance is altered by androgen injections (21).

SUMMARY

White male mice were castrated at 17 to 19 gm. body weight and approximately one month later, separate groups were treated as follows: *a*) injected daily with 1.1 rat growth units (1 mg.) of anterior pituitary growth hormone, *b*) implanted subcutaneously with an approximately 14-mg. pellet of testosterone propionate and *c*) both treatments simultaneously. The mice treated with the growth-hormone preparation showed approximately the same increase in body weight as those treated with the androgens. When the two hormones were administered simultaneously, there was a summation effect on the increase in body weight.

The growth hormone produced a small increase in kidney weight which was not further enhanced by extending the period of treatment. The androgen on the other hand, produced a much greater increase in this organ which was further increased on extending the period of treatment. The effects on the kidney were partially summated when the two hormones were administered simultaneously.

Both hormones increased the total amount of protein and water in the carcass and the organs and decreased the fat of the carcass. The increased amount of protein was similar for both hormones, but the androgen diverted a larger proportion to the kidneys and especially the seminal vesicles and prostates The growth hormone caused a slightly greater increase in water content and a much greater decrease in fat content of the carcass than the androgen. The simultaneous administration of the two hormones produced a summation of the protein anabolic but not the fat catabolic effects.

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EFFECT OF TESTOSTERONE PROPIONATE AND GROWTH HORMONE ON THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE MOUSE[1,2]

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IN PREVIOUS reports it has been demonstrated that testosterone propionate increases the arginase (1–3) and to a lesser extent the 'acid' phosphatase, but decreases the 'alkaline' phosphatase (3–5) of the mouse kidney. These enzymes of the liver are not affected. Since the growth hormone of the anterior pituitary also is a very potent stimulator of protein anabolism (6, 7), it seemed worth while to determine whether this hormone also affected the above enzymes.

PROCEDURE

The mice were those used in the preceding report (8). The enzymes were determined as previously described (1, 2, 5).

RESULTS

Kidney enzymes. The testosterone propionate[3] produced the expected marked increase in arginase (1, 2), the small increase in 'acid' and the marked decrease in 'alkaline' phosphatases (5) (table 1). The arginase and 'alkaline' phosphatase showed no further changes when the androgen treatment was extended to 20 and 34 days. Growth hormone,[4] on the other hand, did not significantly alter the concentration of the enzyme activities, except in the case of the kidney arginase which was moderately increased only in the mice injected for 10 days.

The simultaneous administration of growth hormone with testosterone propionate markedly reduced the ability of the androgen to increase the arginase activity of the kidney, but did not significantly alter the effect on the phosphatases.

Liver enzymes. The hormones either separately or simultaneously administered did not alter the concentration of the various enzymes (table 2).

TABLE 1. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND ANTERIOR PITUITARY GROWTH HORMONE (G. H.) ON ENZYMES OF THE KIDNEY OF CASTRATED MICE

	NO. OF MICE	HORMONE		KIDNEYS	ARGINASE	PHOSPHATASES	
						'Alkaline'	'Acid'
		days	*mg/day*	*mg.*		$(U/gm.)^1$ %	
Control	19			234	50 (30–84)	336 (246–400)	10.3 (8.8–12.9)
T. P.[2]	6	10	0.15	367	+268	−48	0
	9	20	0.16	404	+202	−54	+10
	8	34	0.15	423	+202	−48	+15
G. H.	9	10	1.0	320	+52	+8	+2
	9	20	1.0	304	0	−3	+6
	7	34	1.0	290	+14	−3	+11
T. P.[2] + G. H.	8	20	⎰ 0.16 ⎱ 1.0	464	+100	−37	+14
	8	34	⎰ 0.16 ⎱ 1.0	448	+114	−45	+21

[1] The changes are percentage differences from the control values, which are given in parentheses with the ranges.

[2] Implanted subcutaneously as a 14- to 15-mg. pellet.

TABLE 2. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND ANTERIOR PITUITARY GROWTH HORMONE (G. H.) ON ENZYMES OF THE LIVER OF CASTRATED MICE

	NO. OF MICE	HORMONE		LIVER	ARGINASE	PHOSPHATASES	
						'Alkaline'	'Acid'
		days	*mg/day*	*gm.*		$(U/gm.)^1$ %	
Control	19			0.92 (0.72–1.01)	(16,800) (14,300–18,500)	(8.1) (6.9–9.6)	(18.9) (17.7–20.2)
T. P.[2]	6	10	0.15	0.96	−3	+18	−3
	9	20	0.16	1.04	+8	+5	−13
	8	34	0.15	1.01	+10	+24	−4
G. H.	9	10	1.0	1.19	+10	−2	+18
	9	20	1.0	1.29	+22	+7	+8
	7	34	1.0	1.02	−4	+3	−3
T. P.[2] + G. H.	8	20	⎰ 0.16 ⎱ 1.0	1.24	+5	+26	+15
	8	34	⎰ 0.16 ⎱ 1.0	1.10	+1	+32	+2

[1] The changes are percentage differences from the control values which are given in parentheses with the ranges.

[2] Implanted subcutaneously as a 14- to 15-mg. pellet.

DISCUSSION

It is evident that neither testosterone propionate nor growth hormone decreases the requirement of arginase in the liver while stimulating protein anabolism. On the contrary as the liver is stimulated to increase in size there is a proportionate increase in the enzyme activity. It is impossible at present to reconcile these results with the reported (8) decrease in arginase activity of the liver of the hypophysectomized rat, especially since the decreases observed in either hypophysectomized or normal adult male rats in this laboratory (unpublished) range from 0 to 20 per cent. It is of interest that the 'alkaline' and 'acid' phosphatase activities also increase in proportion to the increase in liver protein.

The two hormones do not show the same effect in their actions on the enzymes of the kidney. The action of the growth hormone is identical to that on the liver. There is a proportionate increase in the enzymes with kidney mass. The androgen, on the other hand, produces a very great increase in the arginase, a small increase in the 'acid' phosphatase and a marked decrease in the 'alkaline' phosphatase activities. Furthermore, the effect of the androgen on the arginase activity is greatly inhibited while the phosphatases are not affected by the simultaneous administration of the growth hormone. Thus, there is a definite difference in the mechanism of the intermediary metabolic processes stimulated by these two protein anabolic hormones. Indeed, there is even a suggestion of antagonism or competition.

SUMMARY

Mice were castrated at 17- to 19-gm. body weight and one month later were implanted subcutaneously with 1) a 14- to 15-mg. pellet of testosterone propionate, 2) injected subcutaneously with 1.1 rat u/day of growth hormone and 3) treated simultaneously with both hormones for 10-, 20- and 34-day periods. Testosterone propionate produced the expected marked increase in kidney arginase, small increase in 'acid' (pH 5.4) phosphatase and marked decrease in 'alkaline' (pH 9.8) phosphatase activities. Growth hormone was ineffective but when administered simultaneously with testosterone propionate, it decreased the arginase-stimulating effect of the androgen to one-half.

The liver enzymes were not affected by either of the hormones. The small increases in liver size were accompanied by proportionate increases in the enzyme activities.

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EFFECT OF ESTROGEN ALONE AND IN COMBINATION WITH TESTOSTERONE ON THE BODY AND ORGAN WEIGHTS AND THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE MOUSE[1]

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IN PREVIOUS studies the effect of testosterone (1) and α-estradiol (2) on the organ weights and enzymes of the castrated mouse have been reported. In this study, the effects of the combination of these two hormones and also the androgen with methoxybisdehydrodoisynolic acid are being reported. In addition studies with two new synthetic estrogens are included.

PROCEDURE

Male mice were castrated at 17- to 19-gm. body weight; one month later the pellets of the various steroids[2] were implanted subcutaneously (3). When both estrogen and androgen were implanted, they were inserted at different sites. Mice of the dba[3] strain were used in all of the studies except at the lower dose for the 16-day experiments and for the studies with 1-methylestrone and 1-methylestradiol for which mice of the Swiss strain from our Bacteriology department were used. This change was necessitated because the dba mice were no longer available.

The food, Rockland rat diet, was fed *ad libitum* and was removed from the cages the day before autopsy. The mice were killed by severing the spinal chord at the base of the skull and bleeding by cutting the blood vessels of the neck. The organs were removed and weighed on a Roller-Smith torsion balance. The weighed kidneys and liver were homogenized and the enzyme activities determined as previously described (3–6).

RESULTS

Rate of absorption of the estrogens. Methoxybisdehydrodoisynolic acid (hereafter designated as MDDA) was absorbed at approximately eight times the rate of α-estradiol (table 2). The solubility of the estrogens or the androgen was not noticeably altered by simultaneous implantation (tables 1, 2). The difference in solubility between the two estrogens was greatly reduced when they were mixed with cholesterol. The MDDA had only about twice the rate of absorption of α-estradiol.

The introduction of the methyl group into α-estradiol, 1-methylestradiol, increased the solubility of the compound approximately fourfold. Similar alteration of estrone, 1-methylestrone, did not produce the same result. It was ineffective (cf. 2).

TABLE 1.   EFFECT OF TESTOSTERONE AND α-ESTRADIOL ON BODY AND ORGAN WEIGHTS OF CASTRATED MICE

	NO. OF MICE	STEROID ABSORBED	BODY WEIGHT		SEM. + PROS.	KIDNEYS	THYMUS
			Final	Change			
10-Day Experiments							
		mg.	gm.	gm.	(mg.), %[1]	(mg.), %[1]	(mg.), %[1]
Control..........................	28		21.5	0.9	(11) (7–13)	(263) (222–295)	(33) (22–53)
Testosterone......................	9	3.3	23.6	2.7	1130	60	−61
Testosterone + α-estradiol.........	5	3.2, 0.7	21.1	0.6	736	58	−61
α-Estradiol.......................	5	0.6	22.0	1.3	191	20	−49
30-Day Experiments							
Control..........................	28		22.3	1.7	(11) (7–13)	(263) (222–295)	(33) (22–53)
Testosterone.....................	9	8.3	24.6	4.4	2860	108	−91
Testosterone + α-estradiol.........	1[2]	7.0, 2.4	20.7	2.4	1690	105	−99
α-Estradiol.......................	6	2.6	20.0	0.1	54	15	−70

[1] The percentage differences are from the average values of the controls which are given with their ranges in parentheses.
[2] Two mice died at 28 days.   The weights of their organs were similar to those of the survivor.

TABLE 2.   EFFECT OF TESTOSTERONE AND VARIOUS ESTROGENS ON BODY AND ORGAN WEIGHTS OF CASTRATED MICE (16-DAY EXPERIMENTS)

	NO. OF MICE	PELLETS	STEROID ABSORBED	BODY WEIGHT		SEM. VES. + PROS.	KIDNEYS	LIVER	THYMUS
				Final	change				
			mg/16 days	gm.	gm.	(mg.), %	(mg.), %	(mg.), %	(mg.), %
Control (dba)	5			23.1	1.4	(16) (14–18)	(257) (232–283)	(1030) (890–1090)	(42) (27–54)
Control (Swiss)	9			23.3	1.5	(12) (8–16)	(250) (217–281)	(960) (760–1060)	(51) (34–73)
Testosterone	5	1	7.0	23.1	2.2	1180	73	−9	−93
	5	1	6.3	24.8	2.8	2310	53	−4	−85
Testosterone + α-estradiol	5	1, 1	6.6, 0.54	21.3	0.4	800	76	−9	−95
	5	1, 1 + 3	7.0, 0.05	26.0	2.8	1970	68	11	−92
α-Estradiol	5	1	.61	20.4	−1.4	0	6	−4	−81
	5	1 + 3	.06	24.8	3.2	150	5	7	−71
Testosterone + MDDA	2[1]	1, 1	5.6, 4.6	15.6	−4.5	375	10	−15	−95
	5	1, 1 + 3	6.9, 0.12	24.2	1.4	1740	49	−3	−99
MDDA	5	1	4.8	15.1	−5.5	−37	−32	−27	−99
	5	1 + 3	0.10	23.4	1.4	41	8	10	−55
1-Methylestrone	4	1	0.33	21.4	0.4	0	−11	−4	0
1-Methylestradiol	4	1	2.2	22.0	1.6	25	4	0	0

[1] Three mice died before the end of the experiment.

Body weight. The mice implanted with pure α-estradiol and MDDA either gained less weight than their controls or lost weight (table 2). The loss in body weight increased with the amount of estrogen absorbed. Thus, MDDA, which was most rapidly absorbed, produced the greatest decrease in body weight. The reduction of the amount of estrogen absorbed by mixture with cholesterol (table 2), permitted α-estradiol to increase the body weight. The MDDA, at the lower dose level, which was, however, twice that of α-estradiol, only maintained the body weight at that of the controls. The simultaneous administration of testosterone exacerbated the urinary retention. Furthermore, 2 out of 3 mice treated for 30 days with testosterone and α-estradiol (table 1) died on the 28th day and 2 out of 4 mice treated for 16 days with testosterone and MDDA died on the day before and the last day of the experiment. One of these had hemorrhage in the large intestine. Moreover, 3 of the mice treated with testosterone and the low dose of α-estradiol (table 2) and 2 treated with testosterone and the low dose of the MDDA had markedly distended bladders at autopsy. The controls and the remainder of the experimental mice had negligible amounts of urine in their bladders.

TABLE 3. EFFECT OF TESTOSTERONE AND α-ESTRADIOL ON THE ARGINASE ACTIVITY OF THE KIDNEY OF CASTRATED MICE

	10-DAY EXPERIMENTS		30-DAY EXPERIMENTS	
	No. of mice	Arginase	No. of mice	Arginase
		(U/gm.), %		*(U/gm.), %*
Control	20	(49) (39-59)	20	(49) (39-59)
Testosterone	6	+490	6	+584
Testosterone + α-estradiol	5	+760	1	+789
α-Estradiol	5	+192	5	+88

Organ weights. The effect of testosterone on the seminal vesicles and prostates was greatly decreased by the simultaneous administration of both α-estradiol and MDDA in all of the experiments (tables 1, 2). The greatest inhibition was observed at the high dose of MDDA (table 2). MDDA and α-estradiol demonstrated a small and the methylated estrogens no renotrophic properties (tables 1, 2). The renotrophic effect of testosterone was not influenced by the simultaneous administration of either α-estradiol or MDDA. The small decrease in liver weight produced by testosterone is equivocal. In other studies no change (7) or an increase (unpublished) has been noted (also cf. 8). The high dose of MDDA decreased the weight of the liver which was partially counteracted by testosterone (table 2). The other experiments showed no or questionable small changes which roughly paralleled the changes in body weight. Testosterone was more effective than the estrogens in diminishing the weight of the thymus. The methylated estrogens were completely ineffective in this respect (tables 1, 2).

Kidney enzymes. Testosterone as expected (1, 3, 4) greatly increased the arginase activity of the kidney (tables 3, 4). The α-estradiol and MDDA (tables 3, 4), but not the methylated estrogens (table 4), also increased this enzyme but not as

TABLE 4.   EFFECT OF TESTOSTERONE AND VARIOUS ESTROGENS ON ENZYMES OF THE KIDNEY OF CASTRATED MICE (16-DAY EXPERIMENTS)

	NO. OF MICE	STEROID ABSORBED	KIDNEYS	ARGINASE	PHOSPHATASES		NITROGEN
					'Alkaline'	'Acid'	
		mg/16 days	mg.	(U/gm.), %	(U/gm.), %	(U/gm.), %	(mg/gm.), %
Control (dba)	5		257	(63) (32–93)	(376) (350–448)	(9.4) (8.1–10.9)	(31.5) (30.6–32.7)
Control (Swiss)	9		250	(33) (12–70)	(391) (270–480)	(9.5) (7.8–10.5)	(29.8) (28.7–30.8)
Testosterone	5	7.0	444	417	−76	3	−5
	5	6.3	381	728	−61	11	−2
Testosterone + α-estradiol	5	6.6, 0.54	453	750	−71	−2	−8
	5	7.0, 0.05	419	839	−55	−1	−4
α-Estradiol	5	0.61	275	35	−26	3	−3
	5	0.06	262	155	−3	5	+1
Testosterone + MDDA	2	5.6, 4.6	282	855	−68	−9	−9
	5	6.9, 0.12	373	903	−48	6	−5
MDDA	5	4.8	176	16	−6	11	4
	5	0.10	271	167	−2	0	−1
1-Methylestrone	4	.33	221	−1	−5	−1	0
1-Methylestradiol	4	2.2	260	4	−5	6	−2

TABLE 5.   EFFECT OF TESTOSTERONE AND VARIOUS ESTROGENS ON ENZYMES OF THE LIVER OF CASTRATED MICE (16-DAY EXPERIMENTS)

	NO. OF MICE	STEROID ABSORBED	LIVER	ARGINASE	PHOSPHATASES		NITROGEN
					'Alkaline'	'Acid'	
		mg/16 days	mg.	(U/gm.), %	(U/gm.), %	(U/gm.), %	(mg/gm.), %
Control (dba)	5		1030	(19800) (16400–22800)	(8.9) (4.8–14.5)	(13.8) (11.0–14.3)	(35.6) (33.1–39.2)
Control (Swiss)	9		960	(17900) (12400–21600)	(8.2) (5.1–10.5)	(13.3) (10.0–14.8)	(33.3) (31.9–34.8)
Testosterone	5	7.0	940	−2	14	4	6
	5	6.3	920	−15	17	9	8
Testosterone + α-estradiol	5	6.6, 0.54	940	−2	52	1	3
	5	7.0, 0.05	1060	−8	16	6	16
α-Estradiol	5	0.61	990	18	92	11	2
	5	0.06	1020	5	16	7	5
Testosterone + MDDA	2	5.6, 4.6	870	152	189	51	−2
	5	6.9, 0.12	920	15	60	20	8
MDDA	5	4.8	760	126	225	44	−6
	5	0.10	1050	27	52	4	5
1-Methylestrone	4	0.33	920	3	−21	6	8
1-Methylestradiol	4	2.2	960	16	−5	9	2

greatly as the androgen. Furthermore, α-estradiol in contrast to testosterone became less effective with continuation of treatment. The greatest increase occurred

after 10 days of treatment (table 3). The simultaneous administration of the androgen and the estrogens always resulted in a summation of their arginase-stimulating properties (tables 3, 4).

Testosterone produced the expected decrease in concentration of 'alkaline' and no effect in 'acid' phosphatase activities (table 2). The total activity of the latter, however, was increased in proportion to the increase in kidney weight. The estrogens were ineffective except for the small decrease produced at the high dose of α-estradiol. Furthermore, the estrogens did not influence the effect of the androgen on these two enzymes.

Liver enzymes. MDDA administered singly or with testosterone (table 5) produced a remarkable increase in the arginase activity at the high dose level and a much smaller increase at the low dose level. No noteworthy changes were evident in the rest of the experiments. The 'alkaline' phosphatase activity also was remarkably increased by MDDA and to a lesser degree by α-estradiol. At the low dose level the MDDA was much less effective and α-estradiol was ineffective. The 'acid' phosphatase was moderately increased only by MDDA at the high dose. The methylated estrogens did not influence the activities of any of the enzymes.

Kidney and liver nitrogen.[4] There were no remarkable changes in the nitrogen (protein) concentration of either the kidney or the liver in any of the experiments (tables 4, 5). In general the change in organ weight was accompanied by a proportionate change in protein content.

<div align="center">DISCUSSION</div>

The opening of the 5 ring in α-estradiol and the addition of the methoxy group to produce MDDA greatly increased its rate of absorption with a concomitant increase in its physiological effects. The introduction of a methyl group in the one position also increased the rate of absorption of α-estradiol, but very greatly reduced its physiological properties. A similar change in the estrone molecule, however, did not change its rate of absorption but did greatly reduce its physiological effects. It would seem that the phenolic A ring plays a very important part in the physiological properties of the estrogens.

The very marked loss in body weight produced by the higher doses of the estrogens is well known (cf. 8). It resembles the results of inanition. The amount of food eaten, however, was not determined.

It is difficult to state whether the toxicity of the estrogens was due to the effects of urinary retention or estrogen imposed starvation. It is of special significance that testosterone instead of protecting against these phenomena actually exacerbated them. The estrogens, on the other hand, had a marked inhibitory effect on the androgenic property of testosterone. Numerous reports (cf. 8) indicate that androgens and estrogens may have a mutually antagonistic or coöperative effect on various parts of the genital tract depending on relative doses.

The decrease in the androgenic activity of testosterone by MDDA and α-estradiol was not accompanied by a similar decrease in renotrophic activity. Thus,

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[4] Katherine Clancy and Carolyn Abbott carried out these determinations.

the combination of the two hormones produced a relationship between the weights of these two organs similar to that produced by androstanediol-3α, 17α and its 17-methyl derivative (1, 7, 9).

The smaller increase in the arginase activity of the kidney at the higher than at the lower dose of α-estradiol and MDDA was probably due to the greater toxicity and loss of body weight. Inanition decreases the kidney arginase-stimulating property of the androgens (4). It is noteworthy that there was a summation of the arginase stimulating property of testosterone and estrogens. This is in contrast to the effect of the anterior pituitary growth hormone which markedly decreases this property of testosterone without affecting the androgen's renotrophic property (unpublished).

The failure of the estrogens to produce any changes in the kidney phosphatases is in agreement with the previous study (2). It is of special significance that the urinary retention was not accompanied by a decrease in the 'alkaline' phosphatase, since this is the first change noted after ligature of the ureters (10). Indeed this enzyme and also arginase nearly completely disappear as the kidneys become non-functional (11).

The remarkable increase in the arginase activity of the liver produced by MDDA is specially noteworthy. This phenomenon was not apparent, moreover, until a very great excess of the estrogen was absorbed—a reverse of the enzyme effect noted in the kidney. The effect of MDDA on the 'alkaline' phosphatase of the liver was even greater than that on the arginase. Furthermore, this increase was noted at the lower dose and also after α-estradiol treatment. A smaller increase in the 'acid' phosphatase was noted only with the high dose of MDDA. The loss in body and liver weight suggests that the increases in these hydrolytic enzymes of the liver may be a reflection of the great demands placed on endogenous protein. Indeed, this may be indicative of the depletion of protein reserves and a breakdown of vital protein structures. The effect on the arginase and 'acid' phosphatase activities was not apparent until the dose of the estrogen was so great that it caused the death of many of the mice.

SUMMARY

Male mice castrated at 17- to 19-gm. body weight were implanted subcutaneously with a pellet of testosterone, α-estradiol, methoxybisdehydrodoisynolic acid (MDDA), 1-methylestradiol and 1-methylestrone. The first two estrogens also were implanted as pellets consisting of one part of estrogen and three parts of cholesterol and simultaneously with a pellet of testosterone. All of the experiments were for 16 days. In addition the testosterone and α-estradiol experiment was performed at 10 and 30 days. The rate of absorption of MDDA was about eight times and that of 1-methylestradiol 4 times that of α-estradiol. The introduction of the 1-methyl group into estrone did not alter its rate of absorption from a pellet. The simultaneous implantation of a pellet of testosterone did not influence the rate of absorption of α-estradiol or MDDA.

MDDA and α-estradiol as pure pellets greatly reduced the body weight in a manner resembling inanition. There was a concomitant retention of urine which was

exacerbated by the simultaneous administration of testosterone. Also deaths occurred only in those mice that were implanted with both testosterone and pure pellets of MDDA or α-estradiol.

The renotrophic property of testosterone was not altered, but the androgenic property was greatly reduced by both α-estradiol and MDDA. The increase in arginase activity produced by testosterone and MDDA or α-estradiol summated when the androgen and either of the estrogens were administered simultaneously. The estrogens did not influence the effect of the androgen on either 'alkaline' or 'acid' phosphatase. MDDA at the high dose produced a remarkable increase in the arginase activity of the liver; α-estradiol was ineffective. MDDA also produced a remarkable increase in the 'alkaline' phosphatase at the high dose and α-estradiol a moderate increase at its high dose. MDDA stimulated a small increase in 'acid' phosphatase at the high dose; α-estradiol was ineffective. 1-methylestradiol and 1-methylestrone were ineffective in all of the above tests.

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EFFECT OF TESTOSTERONE PROPIONATE ON NITROGEN AND CHLORIDE EXCRETION AND BODY WEIGHT OF CASTRATED RATS DURING RECOVERY FROM FASTING[1,2,3]

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SINCE testosterone propionate is known to stimulate protein-anabolic processes (see 1–3 for reviews of literature), it was decided to determine whether this steroid would hasten protein repletion after exhaustion of reserve stores by complete removal of food from adult rats until shortly before death.

EXPERIMENTAL

Animals. The rats were of the Wistar strain from our colony.

Diet. Each rat received 9.5 gm. of the following diet: casein 16.7, sucrose 61.2, hydrogenated vegetable oil 7.4, celluflour 1.8, Wesson's (4) salt mixture 3.7 and dry brewers' yeast (Fleischmann No. 2019) 9.2. The ingredients were mixed in a Hobart mixer and kept at 5°C. Each new batch was analyzed for nitrogen. The average nitrogen content was 2.95 per cent. Supplements of two drops of cod liver oil and one drop of wheat germ oil[4] diluted 10-fold with wesson oil were given each day. Each rat ate all of its food except a small amount of spill, 0.1 to 0.3 gm/day, which was weighed and subtracted from the given amount. Distilled water was provided *ad libitum*.

Procedure. The rats were placed in individual metabolism cages similar to those used by Swift *et al.* (5), figure 2, or metal cages in racks manufactured by the Norwich Wire Works (fig. 3). The temperature of the room was maintained at 78 to 80°F.

The rats were weighed and fed at the same time each day. The injections were made just before or after feeding. In the first experiment (fig. 2), fecal periods of seven days were separated by mixing 0.4 gm. of animal charcoal into the food of the last day of the period. The feces were collected daily and saved until the end of the period in 75 ml. of 30 per cent sulfuric acid in which they disintegrated on warming. Fecal nitrogen was not determined in the second experiment. The urine of each rat was washed from the receptacle and the cage with warm distilled water. Thymol or 3 per cent benzoic acid in 50 per cent ethyl alcohol (6) was used as a preservative. The urine collection periods were two, two and three days each week except in the prefasting period and the last 28 days of *experiment 1* (fig. 2) when the collection periods were extended to seven days.

Nitrogen determination. The micro-Kjeldahl procedure was used for all nitrogen determina

tions except that aliquots of the diets were digested by the macro procedure, made to a given volume and duplicate aliquots distilled by the microprocedure.

Chloride determination. The method of McKittrick and Schmidt (7) was used for the determination of the chloride in the urine and the diet of *experiment 1.*

RESULTS

Determination of length of survival of rats without food. In order to determine how long the rats of our colony could survive without food, 5 adult male rats, varying in body weight from 250 to 348 gm. (fig. 1), from the stock colony were placed in individual cages without food but with tap water *ad libitum* until they died. The body weights were recorded daily. The average survival period was 15.4 ± 1.7 days. The body weight decreased to 45.6 ± 4.1 per cent of the original body weight.

Experiment 1. Prolonged injections of testosterone propionate. The rats were castrated approximately 150 days before the beginning of the fasting period. After 12 days without food one rat died. Death in all probability was due to starvation

Fig. 1. EFFECT OF FASTING on the body weight and the survival of male rats. The rats were placed in individual cages without food but water *ad libitum*. They were weighed daily.
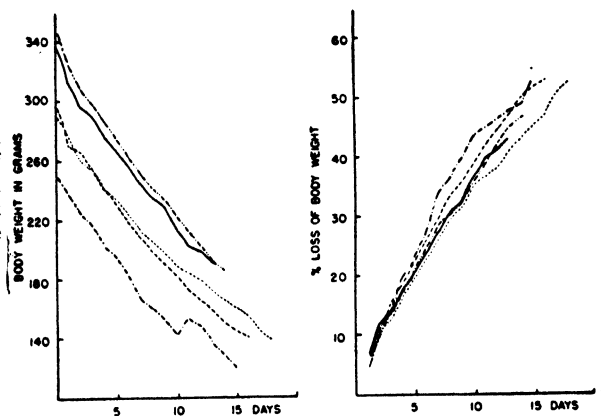
for no gross pathology was observed. The survivors, therefore, were given food At the same time 5 of the rats were injected with 2.5 mg/day of testosterone propionate[5] and the other 4 were injected with 0.1 ml/day of sesame oil.

It seemed at first that the rats injected with testosterone propionate were going to show a much greater increase in body weight than the controls (fig. 2). The initial spurt, however, was of relatively short duration, a maximum difference in body weight of 11 gm. was attained at the 20th and 21st days. It was followed by a much slower rate of gain in body weight so that by the 31st day the control rats had attained the same weight as the injected animals and continued to gain while the treated rats were no longer or only slightly increasing their body weight.

The rats which were to be injected lost an average of 75.4 gm. of their body weight during the period of fasting and the control rats 79.0 gm. The average loss of the two groups was 77 gm. At the end of the experiment the former animals had

[5] The testosterone propionate was provided as perandren by Ciba Pharmaceutical Products, Inc.

regained 68.4 gm. and the latter, 76.8 gm. of their body weight. The increase in seminal vesicles and prostates contributed 5.7 gm. to the body weight of the injected rats. Therefore, the control rats gained 8.4 gm. more than the injected rats, but if the accessory sex organs were discounted they gained 14.1 gm.

The testosterone propionate stimulated an extra nitrogen retention of 0.689 gm. or the equivalent (8) of 20.7 gm. of tissue. Practically all of this retention
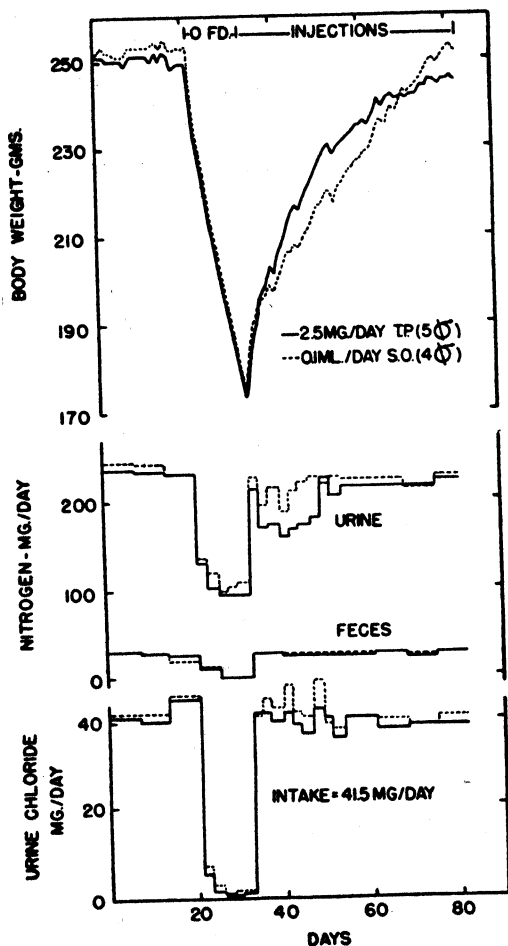
Fig. 2. *Experiment 1.* EFFECT OF TESTOSTERONE PROPIONATE on the recovery of castrated male rats from a 12-day fast. The rats were fed a constant amount of food before and after the fasting period. T.P. = testosterone propionate; S.O. = sesame oil.

occurred during the first 16 days of rehabilitation and accompanied the initial spurt of increase in body weight. Thereafter, the two groups of rats excreted approximately the same amount of nitrogen. At no time, however, did the injected animals lose any of the retained nitrogen. The nitrogen retained was due entirely to excretion of less nitrogen in the urine. The fecal nitrogen was not affected by the injections of the androgen.

The testosterone propionate injected rats excreted a consistently smaller amount

of chloride than the control rats during the periods of nitrogen retention.   It is of
interest that the amount of chloride excreted in the prefasting and the post-retention
periods was identical to that fed.   Also that the amount in the urine rapidly disap-
peared on the withdrawal of food and immediately returned to its previous level
on refeeding.

   Experiment 2.   Post-injection effects.   The rats had been castrated and their
food intake adjusted over a period of 150 days so that they were in body weight
equilibrium for several weeks prior to starvation.   The results during the fasting
and the injection periods were remarkably similar to those obtained in the previous
experiment.   Thus a decrease in dose from 2.5 to 1.0 mg/day did not lower the
effectiveness of the androgen.
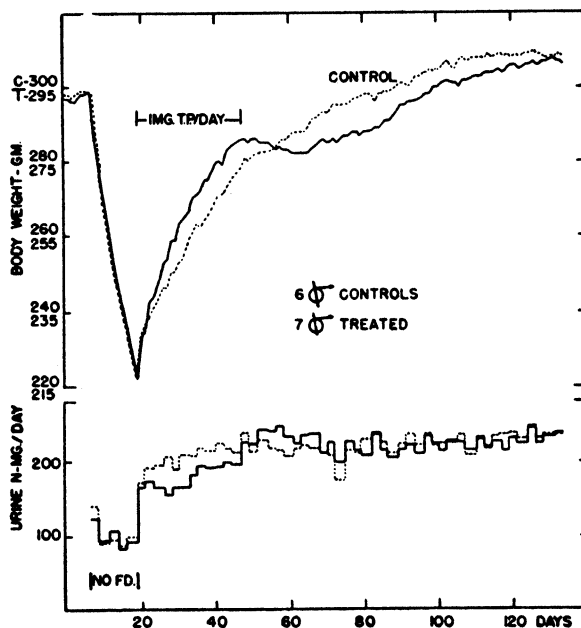
Fig. 3. *Experiment 2.* EFFECT
OF TESTOSTERONE PROPIONATE and
subsequent withdrawal on the re-
covery of castrated male rats from
a 12-day fast.   The rats were fed
a constant food intake before and
after the fast.

   Both groups of rats lost exactly the same amount, 76 gm., in body weight during
the 12 days of starvation.   The testosterone propionate accelerated the restoration
of the body weight so that during the 17th to 20th days the treated rats had gained
10 gm. more than the controls.   Thereafter, the difference in body weights began
to decrease and on the 28th day the injections were stopped.   An immediate cessa-
tion followed by a small loss, 4 gm., in body weight resulted.   Part of this loss in
weight was undoubtedly due to regression of the accessory sex organs (cf. 9-11).
The control rats, on the other hand, continued to increase in weight and soon sur-
passed the treated rats.   After the initial depression in body weight, the treated
rats began to increase in weight again and at a faster rate than the controls.   At
about the 130th day the two groups of rats weighed about the same and their body
weights were progressing at the same rate.

The changes in urinary nitrogen excretion paralleled the changes in body weight. The control rats excreted an insignificantly greater amount, 32 mg., of nitrogen during the 12-day starvation period. The average values for the last 10 days, however, were identical for both groups. The injection of the androgen markedly increased the retention of nitrogen. The greater retention of nitrogen was immediately apparent and continued at the same rate for 14 days, then began to decrease, but was still apparent at the termination of the injections. The androgen stimulated a total nitrogen retention of 630 mg., or the equivalent (8) of 18.4 gm. of tissue, more than the controls during the 28 days of injection. On cessation of injections, the treated rats lost 399 mg. of nitrogen, equivalent (8) to 11.7 gm. of tissue, during the first 35 post-injection days but retained 134 mg., or the equivalent (8) of 3.9 gm. tissue, during the subsequent 16 days. Thus, the injected rats had an overall positive nitrogen balance of 365 mg. or 10.7 gm. of fat-free protoplasm more than the control rats. Since the body weights of the two groups of rats were restored to the same level, the injected rats probably lost an equal amount of fat (12).

DISCUSSION

These experiments demonstrate that testosterone propionate not only accelerates but also increases the degree of protein repletion after exhaustion of reserve stores by starvation. The effect, however, soon 'wears off' in spite of the fact that the animals are in a state of repletion. It is not due to the inhibition of growth stimulators from other endocrine organs. The same phenomenon has been observed in normal, castrated, hypophysectomized and adrenalectomized male rats and in normal and ovarectomized female rats injected with testosterone propionate while on a constant food intake (12).

The cessation of nitrogen retention, however, does not mean that the androgen is no longer effective. Metabolic processes still are stimulated. The accessory sex organs and the kidney continue to grow (12). Since there is no further nitrogen retained, the essential materials for these organs must be obtained directly or diverted from other tissues, e.g. muscle. This preferential stimulation to growth of the accessory sex organs at the expense of other tissues also has been observed in chronically undernourished mice (13) and in fasting dogs (14).

The changes in body weight are similar to those observed in growing young adult normal and castrated rats injected with testosterone propionate (12).

The differences between the observed changes in body weight and those calculated from the nitrogen retained probably are due to a smaller deposition and/or a greater utilization of fat. The chloride values of the urine indicate that no excessive gains or losses in water occurred. Furthermore, testosterone propionate decreases the fat content of the carcass of rats fed either a constant amount or *ad libitum* (12) and mice fed *ad libitum* (12, 15).

The extra nitrogen retained by the androgen-treated rats was much more than could be accounted for by the increase in weight of the seminal vesicles and prostates. In the first experiment, nitrogen equivalent to 20.7 gm. of tissue was retained while the seminal vesicles and prostates weighed 5.7 gm. In the second experiment, there was a loss of much of the retained nitrogen after cessation of injections, but the

equivalent of 10.7 gm. of tissue still was retained at the end of the experiment. This amount of protoplasm was incorporated into tissues other than the accessory sex organs, kidney or liver for these organs rapidly regress on withdrawal of androgen (9-11).

It may be concluded that testosterone propionate accelerates protein replenishment, but the treatment should be confined to the period of nitrogen retention. The androgen has been used successfully in several clinical studies (cf. 1-3).

SUMMARY

Adult white castrated rats were brought into body weight equilibrium and about 150 days after castration were subjected to 12 days of starvation. They were then fed a fixed amount of food and one-half of the animals were injected with testosterone propionate at 2.5 mg/day in the first and 1.0 mg/day in the second experiment. The rats injected with the androgen showed an initially more rapid rate of gain in body weight accompanied by a greater retention of nitrogen. After about the 20th day the body weight began to increase at a much slower rate so that if the injections were continued the weight of the control rats attained and surpassed that of the injected rats. At the same time the nitrogen retention decreased so that there was no difference between the amount of nitrogen excreted by the control or injected rats. On the other hand, if the injections were stopped after 28 days, there was at first a cessation and a loss in body weight accompanied by a loss in nitrogen, then the body weight increased to that of the controls. The nitrogen retained was much more than could be accounted for by the increase in size of the seminal vesicles and prostates. Furthermore, there was a loss of other material, probably fat, for the observed body weight changes were less than calculated from the nitrogen retained. The fecal nitrogen and urinary chloride excretions were not significantly affected by the androgen.

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RENAL CLEARANCE OF ALLANTOIN AS A MEASURE OF GLOMERULAR FILTRATION RATE[1]

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IN A previous study (1) the renal clearance of allantoin in the rat and dog was found to be similar to that of exogenous creatinine. Furthermore, the allantoin clearance of the dog was found to be independent of the blood concentration of allantoin. These two observations of course suggested that the renal clearance of allantoin was a measure of glomerular filtration rate in these two species. This last phenomenon was not surprising after the physico-chemical similarity of allantoin and creatinine was discerned.

In view of the fact that the renal tubule of the rat and dog seemingly could not reabsorb allantoin (a substance naturally present in their blood) from the glomerular filtrate, it seemed possible that the renal tubule of the human subject (whose blood usually contains no allantoin) also might not be able to reabsorb allantoin. Accordingly, the renal clearance of allantoin was measured in a series of normal subjects and in patients suffering from cardio-renal disorders. The clearances obtained also were compared with those of inulin when the latter clearances were done concomitantly in the same subjects. The results of these clearances suggested that the clearance of allantoin in man was a measure of the glomerular filtration rate.

METHODS

Performance of the Clearances. Preliminary studies (2) had indicated that an average blood concentration of approximately 5 to 6 mg. of allantoin per 100 cc. was obtained two hours after the oral ingestion of 10 gm. of allantoin. Furthermore little change occurred in the blood concentration three hours after oral ingestion. Accordingly, each patient received 10 gm. of allantoin dissolved in 500 cc. of orange juice at 8:00 A.M. on the day of the experiment. He was then kept at bed rest until 9:30 A.M. at which time he was catheterized and given 1000 cc. of H_2O by mouth. At 10:00 A.M. the bladder was emptied, washed out with normal saline solution, a blood sample taken and the first urine collection begun. At 10:30 A.M. the bladder was emptied again, washed out with saline solution and a second blood sample was obtained. The second urine collection began immediately after the bladder had been washed and emptied. At 11:00 A.M. the second and final collection period was terminated and a third blood sample was obtained. The allantoin clearance of each subject then was calculated as an average of the clearances obtained for the two collection periods and corrected to 1.73 square meters of surface area.

Subjects upon whom the inulin clearance also was performed were prepared for their allantoin clearance exactly as described above except that at 9:30 A.M. they received 10 gm. of inulin dissolved in 100 cc. of normal saline solution by vein in five minutes, followed by a continuous intravenous infusion of an inulin solution (200 mg. of inulin/100 cc. of normal saline solution) at a rate of 4 cc./minute until 11:00 A.M. The three blood and two urine samples obtained during the clearance periods were analyzed for both allantoin and inulin.

Chemical Determination of Allantoin and Inulin. Allantoin in blood plasma and urine was determined primarily according to the method of Christman *et al.* (3) with an adoption of one of the suggestions of Young and Conway (4).

The actual procedure for small quantities of plasma was as follows: plasma samples were prevented from clotting by the addition of lithium oxalate (1.5 mg./cc. of original blood). Six volumes of a washed yeast-saline suspension (3 gm. of wet washed yeast in 12 cc. of normal saline solution) were added to one volume of plasma, mixed and allowed to ferment for 10 minutes at room temperature with occasional shaking. Plasma proteins then were removed with one volume of 10 per cent sodium tungstate solution and two volumes of N/3 sulphuric acid. Two cc. of protein-free supernatant fluid were pipetted into a test tube, the remainder being reserved for uric acid assay. Equal volumes also of 1) a reagent blank (distilled H_2O), 2) a uric acid correction blank (0.5 mg. % uric acid), 3) a standard solution of allantoin (0.2 mg. % allantoin) and 4) a yeast blank (protein-free supernatant fluid from yeast-saline suspension) were made up at this point and placed into similar test tubes.

To each tube, 0.4 cc. of a N/2 NaOH was added, the contents mixed and the tubes placed in a boiling water bath for seven minutes. They were cooled for three minutes in a bath at 20°C. The N/2 HCl (0.52 cc.) then was added to each tube and mixed and the tubes were immersed in boiling water for exactly two minutes. They were immediately transferred and cooled in a bath at −10° C. for three minutes. Ice-cold phenylhydrazine (0.4 cc. of a 0.33 % solution of phenylhydrazine hydrochloride) was added to each tube and mixed and the reaction allowed to proceed for 15 minutes in a bath maintained at 30°C. After this, the tubes were immersed again in a bath at −10°C. for three minutes. At the end of this time, 1.6 cc. of 10 N/HCl (previously cooled to −10°C.) were added to each tube. Then, without mixing, 0.4 cc. of a potassium ferricyanide solution (1.64 gm. %) previously cooled to 0°C. was added to each tube. A clock was started, each tube mixed by inversion against waxed paper and the contents of each tube poured into respective colorimeter tubes placed and kept in a water bath at 20°C. The color density was read in a Klett colorimeter against green filter No. 54. The entire series of tubes were read in succession, then readings were repeated until at least three had been obtained for the entire series between 6 and 30 minutes after color development had started. Calculations were made from the maximum values recorded.

A separate assay for the uric acid content of each unknown was performed according to the method of Folin (5), in order to determine the correction value.

The usual correction for uric acid was found to be 20 per cent of the value obtained for an equal concentration of allantoin. It should be stressed that for clin-

ical purposes a standard correction value may be assured without individual determinations of plasma uric acid, if the latter is not pathologically elevated.

Determination of the allantoin content of urine samples was carried out exactly as above except that 1) the urine was usually diluted until its allantoin content was

TABLE I. RENAL CLEARANCE OF ALLANTOIN AND INULIN IN NORMAL SUBJECTS AND IN PATIENTS WITH CARDIORENAL DISORDERS

PT.	SEX	AGE	DISORDER PRESENT	U.V.[1]	P.A.C.[2]			A.C.[3]	I.C.[4]	A.C./I.C.[5]
					10:00	10:30	11:00			
A. Normal subjects										
WJ	M.	45	None	13.5	7.4	7.0	7.5	107.0		
HB	M.	22	"	3.7	6.1	7.1	6.8	130.0		
GC	F.	43	"	10.0	5.5	5.1	4.3	137.0		
CR	M.	25	"	11.0	4.8	4.8	6.1	112.0		
GB	M.	25	"	7.5	3.3	4.3	4.1	147.0		
HS	M.	43	"	5.7	6.9	7.7	8.8	123.0	117.0	1.05
DC	M.	32	"	4.3	4.3	4.3	4.8	115.0	96.0	1.20
FT	M.	28	"	8.0	4.6	7.4	4.0	116.0	111.0	1.04
RO	M.	35	"	4.1	8.8	8.4	8.0	118.0	135.0	0.88
Average....................				7.5	5.7	6.2	6.0	123.0	115.0	1.04
B. Patients										
RD	M.	31	Hypertension	1.8	9.2	11.0	8.4	96.0	98.0	0.98
JG	F.	36	"	3.6	6.8	7.4	7.3	119.0	118.0	1.01
DD	M.	53	"	4.1	6.7	7.4	7.1	98.0	104.0	0.94
RF	F.	51	"	5.5	8.3	8.0	8.1	104.0	101.0	1.03
MM	M.	52	"	8.4	8.6	10.8	11.5	113.0	111.0	1.02
AM	F.	24	"	8.3	5.9	6.2	5.5	92.0	104.0	0.88
KK	M.	53	"	3.6	6.9	6.8	6.9	85.0	89.0	0.96
AB	M.	37	"	4.5	4.2	4.5	3.5	105.0	123.0	0.85
RN	M.	14	Coarctation	4.1	6.9	7.4	7.3	84.0	81.0	1.03
ND	M.	53	Nephritis	4.4	6.8	7.0	7.1	97.0	103.0	0.94
VN	F.	36	"	3.5	6.8	7.1	7.1	57.0	45.0	1.26
Average....................				4.7	7.0	7.6	7.3	95.0	98.0	0.99

[1] U.V. equals urine volume in cc/min.   [2] P.A.C. equals plasma allantoin concentration in mg/100 cc.   [3] A.C. equals allantoin clearance in cc/min./1.73 sq. M.   [4] I.C. equals inulin clearance in cc/min./1.73 sq. M.   [5] A.C./I.C. equals ratio of allantoin to inulin clearance in cc/min.

in the range of the standard allantoin solution and 2) preliminary treatment with yeast and deproteinizing chemicals was omitted.

Inulin in plasma and urine was determined according to the method of Hubbard and Loomis (6) modified, however, in that the hydrolysis of inulin was accomplished by the addition of a mixture of two parts of saturated alcoholic HCl and one part of concentrated aqueous HCl alone. Occasionally, turbidity may result following the addition of this mixture to blood filtrates, but this disappears on heating. The

hydrolysis itself was carried out for 10 minutes at 78°C. The resultant color development was read in a Klett photoelectric colorimeter with filter No. 54.

RESULTS

As table 1 *A* indicates, the average allantoin clearance of 9 normal subjects was 123.0 cc/minute (range: 107.0–147.0 cc/min.). The average inulin clearance performed on 4 of these subjects was 115 cc. (range: 96.0–135 cc.). The ratio of the allantoin to the inulin clearance in these 5 subjects was 1.04 (range: 0.88–1.20). Most of the 11 patients studied (table 1 *B*) were suffering from hypertension of the malignant type. Thus the average allantoin clearance was reduced, being 95.0 cc/minute (range: 57.0–119.0 cc.). The average inulin clearance also performed upon these subjects was 98.0 cc/minute (range: 45–123.0 cc.). The ratio of the allantoin to the inulin clearance was 0.99.

DISCUSSION

The similarity in values given by the allantoin and inulin clearances, when performed concomitantly upon both healthy subjects and upon patients suffering from possible reduced renal function, left little doubt that the former type of clearance was a measure of glomerular filtration rate in man just as it had been found to be in the rat and dog (1).

Besides the absence of signs of toxicity following the ingestion of allantoin and the relatively simple and straightforward chemical determination of its presence in blood and urine, its utilization in the estimation of a subject's renal filtration rate offered another advantage. Thus, the solubility of allantoin and its ability to be absorbed from the gastrointestinal tract at an apparently stable rate allowed the opportunity to perform a clearance without the necessity of a continuous intravenous infusion of the substance in a large volume of fluid during the test itself. Furthermore, catheterization is not mandatory since the patient may rise to void his urine during the clearance test.

SUMMARY

The average renal allantoin clearance of normal human subjects was found to be 123.0 cc/minute. When both the allantoin and inulin clearances were performed on the same subject, they were approximately the same. It appears that the renal clearance of allantoin offers a very safe and simple assessment of the glomerular filtration rate in both the normal subject and one suffering from cardiorenal disease.

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SIMULTANEOUS CLEARANCE DETERMINATIONS
IN THE RAT

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SIMULTANEOUS measurement of glomerular filtration rate by inulin clearance, and of renal plasma flow by p-aminohippurate (PAH) clearance, has become a widely used and accepted practice. If such determinations are to have more than relative validity, it is necessary to know that the clearance substances, in required quantities, do not mutually affect the values which they are intended to measure. This study was performed to investigate such interrelationships in the rat.

METHODS

Clearances were measured by the 'tailcutting' method previously reported in detail (1). The clearance substance was injected subcutaneously and blood was obtained from the tail at the start of a clearance period. Urine was collected for one hour and blood was obtained from the abdominal aorta at the end of the clearance period.

The use of groups has been discussed in our earlier reports. In this study 253 normal rats were used, divided into groups of 8 to 10 rats each. The groups were chosen for uniformity in size and sex and specimens of blood and urine within each group were pooled for determination.

Inulin was determined by the method of Alving, Rubin and Miller (2). The PAH was determined by the method of Smith *et. al.* (3).[3] In calculating clearances, mean serum concentrations were used, obtained by assuming a logarithmic rate of fall from the initial to the final concentration, after a single injection. Kidney weight (KW) was measured at autopsy after the clearance period and the clearance was expressed in terms of the measured weight.

RESULTS AND DISCUSSION

The inulin clearance fell steadily as the serum concentration of PAH was increased. At a PAH level of 62.5 mg/100 cc., the inulin clearance was about 65 per

cent of that measured in the absence of PAH (table 1). The PAH clearance (table 2) fell substantially in the presence of increasing serum inulin concentrations. At

TABLE 1. RELATION OF INULIN CLEARANCE TO INCREASING SERUM PAH CONCENTRATION

GROUP NO.	MEAN GM. BW	MEAN MG. KW	URINE VOL. CC/MIN/GM. KW	MIDPOINT SERUM PAH CONC. MG/100 CC.	INULIN CLEARANCE CC/MIN/GM. KW
75A	181	1165	.004	0	1.13
75B	183	1204	.024	0	1.12
69	170	1108	.020	0	1.25
74	152	964	.008	0	1.16
77	161	1111	.014	2.76	1.19
78	144	992	.008	3.94	1.01
54	167	1051	.009	4.01	1.12
53	167	1131	.009	7.58	1.02
66	176	1164	.009	8.16	1.25
84	159	1135	.015	9.29	1.18
61	176	1092	.009	10.40	1.00
86	154	1109	.010	11.32	1.08
71	175	1190	.011	17.65	0.998
67	179	1172	.011	23.9	0.951
68	174	1157	.013	26.2	0.916
82	152	1040	.013	33.5	1.04
90	166	1146	.007	36.3	0.894
99A	173	1172	.014	38.	0.916
58	184	1099	.013	38.2	0.863
101	176	1251	.012	38.6	0.873
76	160	1059	.013	46.5	0.837
143	155	1040	.013	49.1	0.788
81	158	1115	.014	62.5	0.759

TABLE 2. RELATION OF PAH CLEARANCE TO INCREASING SERUM INULIN CONCENTRATION

GROUP NO.	MEAN GM. BW	MEAN MG. KW	URINE VOL. CC/MIN/GM. KW	MIDPOINT SERUM INULIN CONC. MG/100 CC.	CLEARANCE PAH CC/MIN/GM. KW
31	176	1037	.024	0	4.02
30	179	1088	.020	0	3.90
28	181	1162	.016	0	4.67
25	181	1178	.011	0	3.90
23	181	1218	.008	0	4.35
84	159	1135	.015	30.9	3.34
77	161	1111	.014	46.8	3.73
54	167	1051	.009	59.6	3.05
61	176	1092	.009	60.0	2.66
66	176	1164	.009	60.0	3.18
86	154	1109	.010	64.9	3.01
53	167	1137	.009	70.8	3.28
78	144	992	.008	73.8	2.87

an inulin concentration of 73.8 mg/100 cc., the PAH clearance was about 69 per cent of that measured in the absence of inulin.

By plotting the excretion of PAH against the serum PAH concentration, in the presence of a relatively constant serum inulin concentration, a break in the graph was observed at a level of 15 to 20 mg/100 cc. (fig. 1). This presumably defines the level at which tubular saturation is obtained.

The possibility that simultaneously administered substances might interfere with the measurement of renal function has been suggested previously. Klopp, Young, and Taylor (4) found in man that high plasma glucose concentrations depressed PAH clearance and Tm_{PAH}, while high plasma PAH concentrations increased the tubular reabsorption of glucose. In the dog, Houck (5) found that Tm_{PAH} was depressed by high plasma glucose concentrations, but $Tm_{glucose}$ was also depressed by high plasma PAH concentrations, though the effect was not as marked. Crawford (6) recently found that the ratio of exogenous creatinine clearance to inulin or
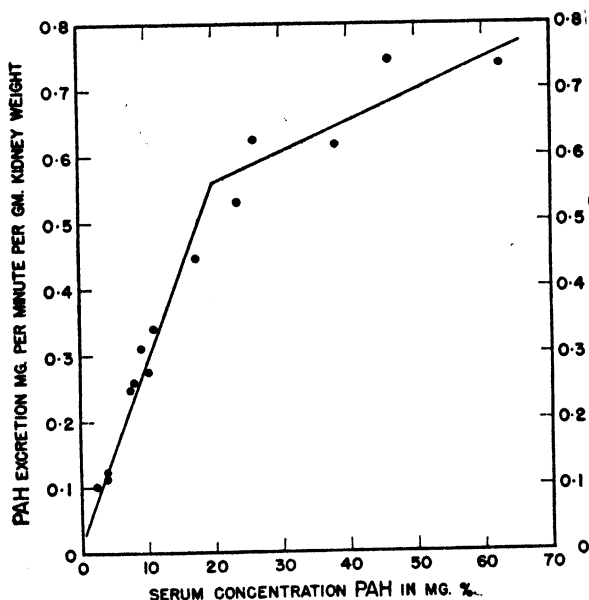
Fig. 1. RELATION OF PAH excretion to serum concentration.

thiosulfate clearances was reduced in man by saturation with diodrast or PAH. Previous reports of determinations on rats (7–11) have not considered the possible interference of clearance substances.

The findings just mentioned concern interferences between processes, both mediated by tubule cells: secretion and reabsorption. Our findings indicate that a substance administered to measure tubular function affects the measurement of glomerular filtration rate in the rat, as well.

The results presented here might be a consequence of several different factors: 1) pharmacological action of the substances used, 2) impurities in the chemical substances and 3) chemical interference in the methods of determination. We can dispose of the last possibility easily. Increasing amounts of PAH did not affect the determination of a standard inulin solution in concentrations simulating those obtained in the clearance studies. Likewise, increasing amounts of inulin did not affect the determination of a standard PAH solution.

It is well known that large doses of PAH produce transient vasomotor effects in man and it is not unreasonable to suppose that similar effects occur with more persistence in the rat. The PAH solution ordinarily obtained contains a brown discoloration, presumably due to oxidation products, offering another source of vasomotor disturbing agents.

We have consistently observed that, after injecting PAH for clearance determinations, the difficulty in obtaining tail blood specimens is directly proportional to the dose of PAH. In addition, after large doses of PAH, the superficial foot veins appear markedly collapsed. It may be noted in table 1 that the reduction in inulin clearance first becomes pronounced as PAH saturation levels are approached, while no effect is perceived at very low PAH concentrations.

Friedman, Polley and Friedman (11) state that tubular saturation for PAH is reached at plasma concentrations of 5 to 6 mg/100 cc. They found no maximum value for tubular excretion of PAH and suggested that the filterable portion of PAH changes at high concentrations.[4] It would seem from their data that none of their determinations were performed at levels sufficiently elevated to obtain tubular saturation.

From these observations, it is concluded that interference between inulin and PAH, as obtained commercially, prevents their simultaneous use in the rat to obtain physiological values for glomerular filtration rate and maximum rate of tubular excretion. Values obtained simultaneously will reflect variations in the concentration of the clearance substances, as well as the conditions of measurement and physiological changes.

SUMMARY

Increasing serum concentrations of PAH reduced the inulin clearance in the rat by one third, an effect that was perceived as the serum PAH concentration approached tubular saturation levels. Increasing serum concentrations of inulin reduced the PAH clearance similarly.

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[4] In a personal communication, Dr. E. Newman suggests that the degree of PAH acetylation may vary in the rat with serum concentration, as it does in man. However, in unpublished data, we have obtained reasonably constant values for TmPAH at serum concentrations higher than 15 mg/100 cc.

HEPATIC AND PERIPHERAL REMOVAL RATES, IN THE DOG, FOR INTRAVENOUSLY INJECTED BROMSULPHALEIN

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IN ORDER to measure hepatic blood flow or maximal hepatic transfer capacity quantitatively, the substance employed must be handled exclusively by the liver. Extra-portal organs should neither metabolize this substance nor remove it from the blood stream. Although there is little experimental evidence that bromsulphalein (BSP) is removed from blood solely by the liver, Bradley, Inglefinger, and associates (1, 2) use this dye for estimating hepatic blood flow and Mason *et al.* (3, 4) employ BSP for measuring maximal hepatic transfer capacity ('Lm'). Both groups of workers base their results on the contention that extra-hepatic and extra-portal sites of BSP removal can be 'saturated' so that they no longer participate in clearing blood of the dye.

In a previous publication (5), we reported that bromsulphalein could not be used to estimate hepatic blood flow, since the dye is not solely disposed of by the liver and portal organs. This paper presents additional evidence that BSP as now used is unsuitable for measuring either hepatic blood flow or hepatic maximal transfer capacity.

METHODS

Two types of experiments were employed.

a) In order to determine whether the peripheral tissues of the dog could be 'saturated' with respect to their ability to remove BSP, eviscerated-hepatectomized-nephrectomized dogs were given a priming dose of the dye[2] (5–7 mg./kg.) followed by a constant intravenous injection (0.1–0.7 mg/kg/min.) of the material for periods up to three hours. Serial serum BSP levels were determined and maintained above a concentration of 20 mg. per cent. Except for the operative manipulations, conditions were similar to those employed by Mason to determine 'Lm' in intact dogs. Apparent 'Lm' values were calculated according to this investigator (4). For our calculations, we assumed 5 per cent of the intact body weight to be plasma.

b) The simultaneous rates of disappearance from the blood of BSP and Evans Blue after a single intravenous injection into eviscerated-hepatectomized-nephrectomized dogs were followed for 60 minutes. These animals received 5 to 10 mg. of Evans Blue and 40 to 75 mg. of BSP immediately after evisceration. It was hoped

that this type of experiment would aid in evaluating capillary permeability since increased permeability might account for the disappearance of BSP. Serum BSP concentrations and evisceration-hepatectomy-nephrectomy were performed as previously described (5). A correction was made in determining the BSP level for the absorption of light at 580 mμ due to Evans Blue.

TABLE 1. DISAPPEARANCE RATE ('PM') OF BSP IN EVISCERATED-HEPATECTOMIZED-NEPHRECTOMIZED DOGS RECEIVING A CONSTANT INTRAVENOUS INJECTION OF THE DYE

DOG	WEIGHT	TIME	TOTAL BSP INJ. DURING TIME PERIOD INDICATED	SERUM LEVEL	SERUM BSP		BSP DISAPPEARING	
					Total circulating	Circulating incr.		
	kilo	min.	mg.	mg. %	mg.	mg.	mg/min.	mg/kg/min.
1	10.0	0*						
		30	266	51.0	255			
		70	288	100.0	500	245	1.07	0.107
		100	216	128.0	640	140	2.53	0.253

* 50 mg. of BSP as priming dose at time 0, followed by 7.2 mg/min. for duration of experiment

2	7.5	0*						
		25	87.5	30.0	112.5			
		55	63.0	44.0	165	52.5	0.33	0.044
		82	57.7	55.0	206	41	0.62	0.082
		117	73.5	65.0	244	38	1.03	0.137
		147	63.0	73.0	274	30	1.1	0.147
		177	63.0	78.0	293	19	1.47	0.183

* 35 mg. of BSP as priming dose at time 0, followed by 2.1 mg/min. for duration of experiment

3	11.0	0*						
		27	84	18.8	103			
		62	44	21.2	117	14	0.86	0.078
		92	38	26.3	145	28	0.33	0.03
		122	38	28.8	158	13	0.83	0.076
		152	38	32.6	179	21	0.57	0.05
		182	38	35.0	193	14	0.80	0.073

* 50 mg. of BSP as priming dose at time 0, followed by 1.26 mg/min. for duration of experiment

RESULTS AND DISCUSSION

a) Effect of a constant intravenous injection of BSP on total circulating quantity of dye in eviscerated-hepatectomized-nephrectomized dogs. After a priming dose and constant intravenous injection of BSP, the results demonstrate a continual disappearance of some of the circulating dye. This should not have occurred had the peripheral depots been 'saturated'. Table 1 shows that when apparent 'Lm' values are calculated according to Mason, results are obtained which indicate that between 0.03 and 0.25 mg/kg/min. of dye disappear.

b) Comparison of simultaneous rates of disappearance of BSP and Evans Blue

after a single intravenous injection into eviscerated-hepatectomized-nephrectomized dogs. Bromsulphalein disappeared from serum more than three times as rapidly as Evans Blue which was injected at the same time into three dogs (fig. 1). Two of the three animals were followed with hematocrit values; these dogs showed a volume of packed red blood cells of 40 and 42 respectively immediately after evisceration. Sixty minutes later, the corresponding values were 39 and 42.

Our results indicate that bromsulphalein is removed from blood in the absence of the liver and portal organs. Although it is claimed that extra-hepatic and extra-
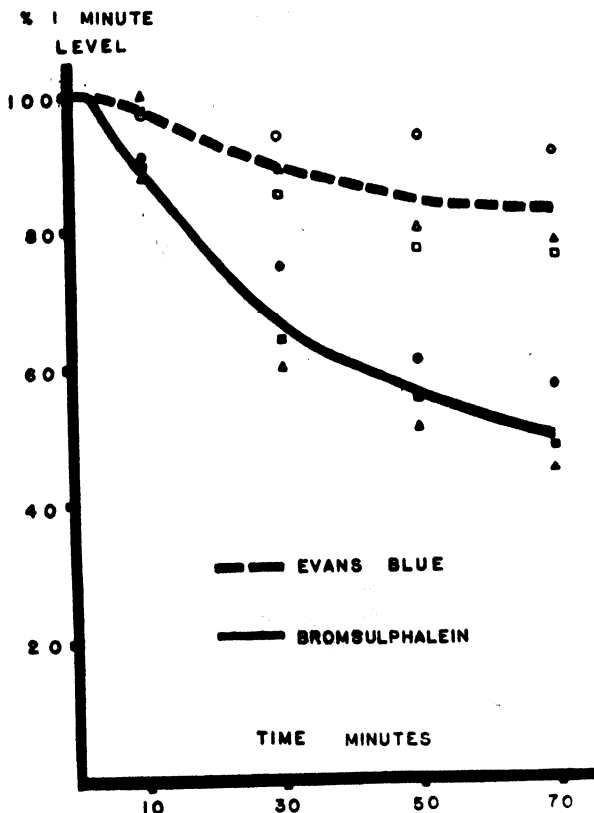
Fig. 1. SIMULTANEOUS RATES OF DISAPPEARANCE OF BSP AND EVANS BLUE after a single intravenous injection of both dyes at time o into eviscerated hepatectomized-nephrectomized dogs. The various points were determined by assigning the one-min. serum level a value of 100%.

Dog 1 (circles) 15 kg. Evans Blue = 10 mg. I.V. at time o; one-min. serum level = 1.70 mg. %; BSP = 75 mg. I.V. at time o; one-min. serum level = 11.0 mg. %.

Dog 2 (squares) 12.5 kg. Evans Blue = 7 mg. I.V. at time o; one-min. serum level = 1.75 mg. %; BSP = 75 mg. I.V. at time o; one-min. serum level = 15.25 mg. %.

Dog 3 (triangles) 8.5 kg. Evans Blue = 5 mg. I.V. at time o; one-min. serum level = 1.75 mg. %; BSP = 40 mg. I.V. at time o; one-min. serum level = 15.25 mg. %.

portal sites of BSP removal can be 'saturated' with the dye and therefore rendered incapable of extracting more of the material from blood, we have not been able to demonstrate this 'saturation' in the eviscerate preparation. In attempting to achieve 'saturation', we carried out our experiments with serum concentrations at least five times the 'saturating' level advocated (4). Mason believed that a serum BSP level of 4 mg. per cent was more than sufficient to 'saturate' extra-portal sites for the dye's removal, but we were unable to 'saturate' these sites with serum levels as high as 100 mg. per cent. On the contrary, our results actually give some indication that the percentage of BSP lost from serum at high concentrations is greater

than at low ones. This could be expected if an active peripheral removal mechanism was present. It thus becomes possible to calculate the 'Pm' (maximal peripheral transfer capacity for BSP). This capacity appears to vary from animal to animal and bears some relationship to serum concentrations of dye. Our results for 'Pm'— varying between 0.03 and 0.25 mg/kg/min.—are one-tenth to one-half of the values Mason reported for 'Lm' in intact animals. These results are based on calculations which assign 5 per cent of the body weight to plasma volume. Since the results on the Evans Blue experiments show the plasmavolume of the eviscerate preparations to be closer to 4 per cent of the intact weight, actual 'Pm' values are even greater than calculated.

Evans Blue disappeared in the eviscerate-hepatectomized-nephrectomized preparation at an average rate of about 15 per cent in the first hour after injection. BSP, infused at the same time as the Evans Blue, was removed from plasma so rapidly that only 50 per cent remained 60 minutes after its injection. Since the reported values for the disappearance of Evans Blue in intact animals are given as between 3 and 12 per cent in the first hour after injection, (6–8), it becomes apparent that the disappearance of BSP cannot be explained on the basis of increased capillary permeability. Rather the results suggest active peripheral removal of injected bromsulphalein. Further proof of normal permeability is given by the constancy of the hematocrit values.

CONCLUSION

Bromsulphalein disappeared from the blood of eviscerated-hepatectomized-nephrectomized dogs more than three times as rapidly as Evans Blue, which was injected intravenously at the same time. No evidence for excessive loss of BSP through the capillaries could be demonstrated in the eviscerate preparation. It was possible to estimate 'Pm' (maximal transfer capacity of the periphery for bromsulphalein), since it was impossible to 'saturate' extra-hepatic and extra-portal sites of bromsulphalein removal with serum levels as high as 100 mg. per cent. The rate of removal of intravenously injected BSP, being the sum of both portal and peripheral removal mechanisms, invalidates the use of the dye for measuring either hepatic blood flow or of 'Lm'.

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MECHANISMS OF DESOXYCORTICOSTERONE ACTION: IV. RELATIONSHIP OF FLUID INTAKE AND PRESSOR RESPONSES TO OUTPUT OF ANTIDIURETIC FACTOR

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IMPLANTATION of desoxycorticosterone acetate (DCA) pellets in rats is followed by a progressive elevation of fluid intake, complete in approximately 10 days (1, 2). The new intake level is related to that of controls of the same weight by a ratio fixed by dosage and degree of supplementary sodium chloride administration (2). Since fluid balance in the normal animal is presumed to represent an equilibrium between the adrenal cortex and the posterior pituitary (3), it was considered that the upward disturbance of this adjustment by an excess of desoxycorticosterone should evoke a counter-balancing output of antidiuretic factor.

The association between antidiuretic and pressor factors in posterior pituitary extracts suggested, furthermore, that the increase in blood pressure which occurs subsequent to DCA-induced elevation of fluid intake (4) might represent the pressor aspect of a combined posterior pituitary response. These possibilities were explored by comparison of the changes produced by DCA in fluid intake and blood pressure with the output of antidiuretic factor in the urine.

PROCEDURE

The experimental animals for this study consisted of 40 immature rats of the Sprague-Dawley strain. All animals were kept in separate cages and were fed on Purina Laboratory Chow. Consumption of food and fluid was unrestricted. After a 2-week period of observation the animals were divided into two groups matched as to weight, sex and fluid intake. One group was given water as the drinking fluid while the other received isotonic (0.86%) salt solution.

Single 20 mg. DCA pellets with an absorption time of four to six months were implanted subcutaneously in 10 animals of each group, using ether anesthesia. The remaining animals were sham operated. Fluid intake was measured daily and weight weekly. Repeated blood pressure determinations were made by a modification of the tail method (5) commencing at the 12th week, the time at which maximum blood pressure elevation may be expected under the conditions stated (2).

Five weeks after pellet implantation, 48-hour urine collections were initiated and were repeated at approximately two-week intervals for five months. The collections were made under toluene, sufficient one per cent acetic acid being added to make the urine weakly acid. The first 24-hour sample was refrigerated until the

second day's collection was completed.   A total of 30 collections from the salt-treated group and 10 from the water-treated group were assayed for antidiuretic activity.

Preparation of Urines for Assay.   The procedure adopted for the preparation of the urine concentrates was similar to that used by previous investigators (6).   The urines were dialyzed against running tap water until chloride free (5–6 hr.).   The samples were then evaporated to dryness in shallow vessels at 34° to 38°C.   Evaporation required approximately 48 hours, the time varying somewhat with the amount of urine.   When amounts greater than 200 cc. were collected, aliquots were used.

On the day of assay the dried residues were dissolved in distilled water to form solutions from 4 to 10 times as concentrated as the original urine.   These solutions were centrifuged and the supernatent solution decanted in order to obtain a clear fluid for injection.   The *p*H was adjusted to lie between 7.5 and 8.5.   No samples were stored, either wet or dry, for periods longer than five days.

METHOD

The rats used for assay of urinary antidiuretic factor output were adult males of the Sprague-Dawley strain, weighing 200 to 300 gm.   The animals were maintained in Purina Fox Chow and were given water *ad libitum.*

The assay was performed by a modification of the method of Burn (7).   Measurements of antidiuretic activity were based on the change in rate of renal excretion of water in hydrated rats after administration of the test substance.   The activity of the urine concentrates tested was estimated by comparison wit'ı the delay in excretion produced by standard amounts of pituitrin in the same assay animals.   Pituitrin (*S*), Parke, Davis and Company, containing 20 I.U./cc., was used as the reference standard and diluted with distilled water to final concentrations of 5, 10 or 15 mU/cc.

Precautions were taken to obtain as constant a response as possible.   No groups were used for assay purposes more than once weekly.   Animals which developed skin lesions at the injection site were discarded until healing had occurred.   This complication developed more frequently in the animals injected with pituitrin than in those which received urinary concentrates or water.   Whenever the total urine collection permitted, each concentrate was tested on two groups of animals and the results of multiple concentrations compared as an internal check on the reliability of the assay method.

On the day preceding assay all food was removed from the animals at 5 P. M.   On the next day a preliminary dose of water amounting to $2\frac{1}{2}$ per cent of body weight was administered orally three hours before assay time.   At the time of assay the animals were hydrated by oral administration of water to the extent of 5 per cent of body weight.   Immediately following hydration the urine concentrate to be assayed was injected subcutaneously in a volume amounting to 1 cc/100 gm. of body weight.   The control animals were injected with an equal amount of water.

Following injection, the urine output was measured at 15-minute intervals.   The period from the midpoint of the time taken to inject the assay group (av. time 6 min.), until the excretion of 50 per cent of the total amount of fluid administered at the time of assay, was used to calculate the antidiuretic activity of the urine concentrate under test.

RESULTS

Analysis of a total of 90 control measurements of the normal 50 per cent excretion time and of the response to pituitrin indicated that the end point value was not sig-

TABLE 1. EFFECTS OF HYDRATION AND POSTERIOR PITUITARY EXTRACT ON URINARY OUTPUT. RELATIONSHIP TO WEIGHT OF ASSAY ANIMAL

| PROCEDURE	WEIGHT RANGE		NO. OF ASSAYS	AV. 50% EXCRETION TIME	STANDARD DEVIATION	t VALUE (LOWER HALF VS. UPPER HALF OF WT. RANGE)
	fraction	*gm.*		*min.*	*min.*	
Hydration, 5% of Body Wt.	Lower 1/2	156 to 244	27	122	±12.6	0.66
	Upper 1/2	245 to 338	27	120	±10.6	
	Total	156 to 338	54	121	±11.6	
Hydration + Pituitrin 5 mU/100 gm.	Lower 1/2	195 to 246	8	172	±34.5	1.04
	Upper 1/2	247 to 276	8	156	±26.2	
	Total	195 to 276	16	164	±30.8	
Hydration + Pituitrin 10 mU/100 gm.	Lower 1/2	169 to 242	9	196	±25.6	−0.29
	Upper 1/2	243 to 326	9	200	±33.2	
	Total	169 to 326	18	198	±28.6	.
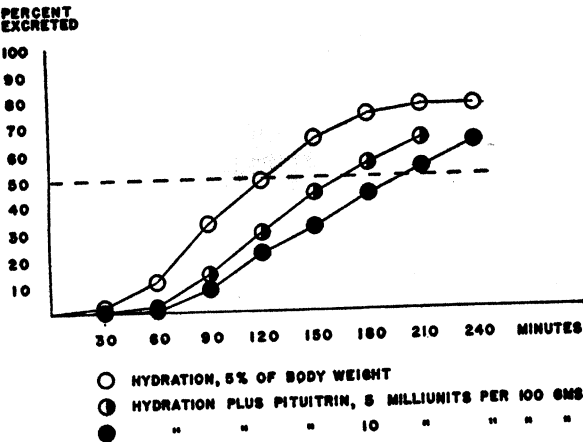
Fig. 1. URINARY EXCRETION RATES following hydration and pituitrin administration. Each composite curve is based on 10 representative assays.

nificantly influenced by the weight of the assay animals over the ranges studied (table 1). The average delay in excretion was directly proportional to the pituitrin dosage over a range of 0 to 10 mU/100 gm. of body weight (table 1, fig. 1). The

concentration of antidiuretic factor in the urine of the test animals was estimated on the basis of this relationship.

TABLE 2.   ANTIDIURETIC FACTOR EXCRETION IN RATS FOLLOWING IMPLANTATION
OF DCA PELLETS

GROUP	DRINKING FLUID	ANTIDIURETIC FACTOR EXCRETION MEAN AND S.D., MU. OF PITUITRIN	FLUID INTAKE (RATIO TO WATER-FED CONTROLS)	BLOOD PRESSURE, MEAN AND S.D., MM.HG
Controls	Water	1.6 ± 2.4	1.00	131 ± 13
	NaCl 0.86%	8.0 ± 4.9	1.39	120 ± 8.6
One 20 mg. DCA implant	Water	5.0 ± 2.6	1.21	150 ± 21
	NaCl 0.86%	20.8 ± 8.7	3.31	196 ± 11

Fig. 2. RELATION OF URINARY ANTIDIURETIC FACTOR OUTPUT to fluid intake and blood pressure. Antidiuretic output is expressed in milliunits of pituitrin. Fluid intake is stated as a ratio, the value for water-fed controls being taken as 1. Blood pressure is given in mm. Hg.
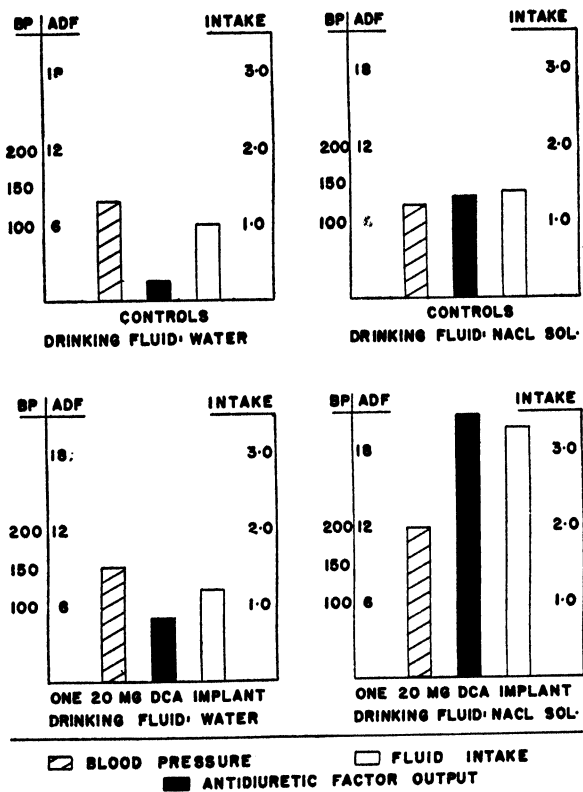
Inspection of the antidiuretic outputs of the various experimental groups (fig. 2) indicated a direct proportionality to fluid intake, not only in the implanted animals but in the saline-fed controls as well. The increase in these two values when saline administration was combined with desoxycorticosterone implantation was greater

than the sum of the increases produced by each procedure separately (table 2). The magnitude of the output, like the degree of intake elevation, was not shown to vary significantly throughout the observation period when once established.

In contrast, no immediate relationship between antidiuretic factor and blood pressure was evident. Water-fed animals which developed significant hypertension ($t = 3.04$) as a consequence of desoxycorticosterone implantation excreted somewhat smaller amounts of the factor than did salt-treated controls in which blood pressures remained normal.

SUMMARY

Subcutaneous implantation of desoxycorticosterone in rats was followed by increases in fluid intake, blood pressure and urinary output of antidiuretic factor. Substitution of isotonic salt solution for drinking water in animals not treated with desoxycorticosterone produced an increased output of antidiuretic factor unaccompanied by significant blood pressure elevation. The urinary output of antidiuretic factor was proportional to the degree of elevation of fluid intake, whether increased intake was produced by salt administration alone, desoxycorticosterone alone, or a combination of both. The evocation of antidiuretic factor excretion by desoxycorticosterone would appear to be a consequence of the disturbance in fluid exchange. A direct relationship of this factor to the development of hypertension was not established.

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WATER AND ELECTROLYTE BALANCE IN DOGS INTOXICATED WITH NITROGEN MUSTARD

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THE nitrogen mustards comprise a series of chemically related *bis* (2-Chloroethyl)amines which exhibit unique biological actions resembling in general the effects of penetrating radiations. Such properties include elicitation of chromosomal changes and genic mutation and inhibition of mitosis or selective destruction of proliferating cells and tissues (1, 2). The latter action has been found therapeutically useful in the treatment of malignant lymphomas (3-5).

Although the effects of the nitrogen mustards on certain of the actively proliferating tissues of the mammalian organism have been clearly described, the mechanisms of the lethal actions of the agents are still obscure. Thus, dogs fatally intoxicated with nitrogen mustards die within three to five days and exhibit pathological lesions in the myeloid and lymphoid elements of hematopoietic organs and in the mucosa of the gastrointestinal tract. Death is not the result of agranulocytopenia. However, the degenerative changes in the gastrointestinal tract may be directly concerned with the mechanism of lethal action. Anorexia, vomiting and diarrhea contribute to marked losses of water and electrolyte which frequently result in a reduction of extracellular and plasma fluid volumes. The associated oligemia may be the proximate cause of terminal circulatory failure (6).

Inasmuch as disturbances of water and electrolyte metabolism are manifested during the course of fatal nitrogen mustard intoxication, studies correlating changes in both intracellular and extracellular ions were undertaken in dogs. It was hoped thereby to determine whether the losses of electrolyte and fluids sustained as the result of intoxication are sufficient to account for the fatal syndrome.

EXPERIMENTAL PROCEDURE

Twenty-three dogs were used. The experiments were divided into three series according to the agent administered and the route of administration. Animals of Series I received *tris* (beta-chloroethyl)amine percutaneously in the dose of 20 mg/kg. The free amine, freshly prepared from the hydrochloride salt, was spread as completely as possible over the shaved skin of the lumbo-sacral region. *Dogs 7* and *8* were muzzled for three hours following application of mustard. At the end of this

time their skins were decontaminated by thorough washing with soap and water. The remaining animals were muzzled for four hours and no decontamination procedure was employed.

Series IIA and IIB were originally undertaken to elucidate a possible rôle of the nervous system in the systemic effects of lethal doses of nitrogen mustards. For this purpose the highly reactive, quarternary transformation product of methyl-*bis*(2-chloroethyl)amine (1, 7), namely cationic methyl-2-chloroethyl-ethylenimonium, was employed. The effect of this agent in animals receiving intracarotid injections of 0.25 to 1.0 mg/kg. was compared with its actions in animals receiving similar doses intrajugularly. However, only *dogs 12, 13* and *16* receiving 1.0 mg/kg. and *14, 15, 17* and *18* receiving 0.5 mg/kg. were used in electrolyte balance studies.

Animals used in the balance studies were fasted but allowed free access to water. All underwent a preliminary period of fasting prior to the time of administration of nitrogen mustard. In the case of *dogs 1, 2, 5,* and *6*, this period was of 24 hours and, for the remaining animals, of 72 hours' duration.

Fluid excretions were collected under mineral oil in the presence of H_2SO_4 and thymol. The cages were washed daily and the collected excreta and washings made up to a known volume. The samples were then filtered, excess thymol added and portions stored under refrigeration until completion of analysis. Since the first 4 animals studied were not catheterized, daily fluid collections in these dogs *1, 2, 5, 6* did not represent true 24-hour samples. Subsequent animals were catheterized 24 hours before the administration of mustard, immediately prior to its administration, 48 hours later, and daily thereafter.

Venous blood samples were obtained at the end of each period of fluid collection. With the exception of *dogs 1, 2, 5, and 6* strict anaerobic technic was employed. A small portion of each blood sample from controls and animals of Series I was heparinized and reserved for the determination of plasma specific gravity. The remainder was allowed to clot and all other analyses were performed on serum.

METHODS OF ANALYSIS

Fluid excretions were analyzed for total nitrogen by the micro-kjeldahl procedure, for sodium by the method of Butler and Tuthill (8), for chloride by the Eisenmann modifications of the Volhard method as described by Peters and Van Slyke (9) and for phosphorus by the method of Fiske and Subbarow (10). Potassium was determined by modifications of the procedures of Shohl and Bennett (11), Consolazio and Talbott (12) and Harrison and Darrow (13) as follows: suitable aliquots were dried and ashed at 500°C. in vycor crucibles (30 cc. capacity). To the ash was added 0.2 cc. of 20 per cent H_4PtCl_6 followed by 5.0 cc. of absolute ethanol. The precipitate which formed was stirred into a homogeneous suspension and an additional 1.0 cc. of absolute ethanol used to rinse the stirring rod. After standing for 30 minutes the alcoholic solution was separated from the precipitate by means of an inverted porcelain filter. The washing with absolute alcohol was repeated twice. The precipitate remaining in the crucible and on the face of the filter was then dissolved in a few cc. of warm distilled water and the solution was transferred through the filter by reduced pressure into a large test tube. The remainder of the procedure follows that described by Harrison and Darrow (13). Using this method 0.01 mEq. of potassium could be determined with an error of one per cent.

The serum was analyzed for sodium, potassium and chloride by the same methods described for urine as adapted to serum. CO_2 was determined manometrically by the method of Van Slyke and Neill (14). Non-protein nitrogen was determined in trichloroacetic acid filtrates by the micro-kjeldahl method. Serum water was measured by difference in weight of samples after drying to constant weight at 105°C.

Plasma protein was calculated from specific gravity (15) which was determined by the falling-drop method of Barbour and Hamilton (16). Serum protein was calculated as 6.25 times the difference between total serum nitrogen and non-protein nitrogen.

CALCULATIONS

Changes in quantities of extracellular water and electrolyte and balance of intracellular sodium as well as the amount of potassium lost in excess of protein catabolized were calculated using the methods of Elkinton and Winkler (17) and Darrow (18) from data provided by the balance studies. For the convenience of the reader a list of the terms employed follows:

E_{Cl}, volume of extracellular water assumed initially equal to 25 per cent of body weight and calculated thereafter from the balance of chloride and serum chloride concentrations;

b_{Na}, b_{Cl}, b_K, balances of sodium, chloride and potassium, respectively;

b'_{Na}, balance of intracellular sodium;

b'_K, balance of potassium in excess of protein lost.

In order to emphasize the adverse effect of intoxication with nitrogen mustard additional calculations were employed to proportionate losses of sodium and chloride to initial quantities of the extracellular electrolytes present in the body. Initial quantities of extracellular sodium and chloride were estimated as the products of initial E_{Cl} and initial concentrations of sodium and chloride in extracellular water respectively. Concentrations of the ions in extracellular water were derived by correction of serum concentrations for serum water and an assumed Donnan factor of 0.96. Balances of sodium and chloride could then be expressed as percentages of the estimated initial quantities of the two electrolytes.

Calculations of potassium released in excess of protein catabolized (b'_K) assume special importance in view of the marked deficit in this cation observed in intoxicated animals. Calculations were based on the assumption that potassium and nitrogen resulting from cell catabolism are excreted in the urine in the relative proportions present in skeletal muscle, i. e. 3 mEq./gm. N (18, 19).

Balances do not include corrections for losses in blood drawn or for changes in serum potassium since these quantities were considered insignificant by comparison with the large amounts of electrolyte excreted during the course of the experiments. Total protein catabolized was calculated from nitrogen excreted corrected for relatively minor changes in non-protein nitrogen content of body fluids.

CLINICAL COURSE

The control animals (*1, 2, 3* and *4*) were unaffected by the short period of fast. In the three series of experimental dogs, survival time of fatally intoxicated animals ranged from three to six days. In the case of the dogs of Series IIA and IIB no distinction in toxicity could be made between the two routes of administration of the ethylenimonium compound. Of the 6 animals receiving more than 0.5 mg/kg. by either the jugular or carotid route none survived. Following intrajugular administration one of 3 survived the dose of 0.5 mg/kg. and one survived 0.25 mg/kg. After intracarotid injection 2 of 4 survived 0.5 and 1 of 2 survived 0.25 mg/kg.

The clinical course of the three groups of animals was in general similar. The typical listlessness and depression which accompany nitrogen mustard intoxication were equally evident in all animals. Vomiting and diarrhea were observed in all of the three series. In Series I, all animals vomited within four to six hours after application of the amine mustard. The extent of vomiting in this group varied greatly in different animals and, inasmuch as vomitus was collected together with the urine, no accurate measure of its volume could be made. In almost every instance vomiting had ceased by the second day. On the other hand, emesis occurred within one to two

hours after injection in the dogs of Series IIA and IIB and continued intermittently until the animals died. Although no quantitative comparison was made of the extent of vomiting in Series I relative to the extent in Series IIA and IIB, there was little doubt that emesis was more severe in the two last mentioned series.

Diarrhea also complicated the experiment somewhat in that liquid feces contaminated the urine. However, diarrhea was never observed during the first 48 hours after administration of the amine mustard and was a variable feature thereafter. Of dogs in Series I, only *7, 9, 10* and *11* excreted a significant amount of watery stool. Diarrhea was evident in all the animals of Series IIA and IIB after 48 hours and was also more severe than in the animals of Series I. Watery stools undoubtedly contributed a large portion of the excretions during the latter part of the balance studies of Series IIA and IIB.

Aside from the vomiting and diarrhea there were no outstanding signs of intoxication. The animals became progressively weaker and were usually prostrate for several hours before death. *Dog 8* was less affected by the dose of mustard employed presumably because it had been decontaminated with soap and water three hours after poisoning. It is possible that the animal might have survived if it had not been killed to obtain tissue for analysis before it was appreciated that its behavior was at variance with that of the other animals.

The clinical course of the surviving animals of Series IIA and IIB was not entirely uneventful. For example, *dogs 15* and *18* developed diarrhea and appeared weak during the first four days after intoxication. Following this the animals recovered and ate ravenously when offered food at the termination of the experiment.

Gross pathological examinations were performed on all animals of Series I at the time of death. The findings were in keeping with those that have been reported by others. In all instances the gastrointestinal tract was examined throughout its length. *Dogs 5* and *6* revealed no hemorrhagic lesions, a finding confirmed by microscopic examination.[3] *Dogs 9, 10* and *11* showed a severe enteritis with no signs of frank hemorrhage. Although there were quantitative differences with respect to the severity of the lesions in the three dogs, the pattern of the intestinal lesions in regard to the areas most seriously affected was similar. Thus, gastritis was seen in all animals, the duodenum was little affected, enteritis was present throughout the jejunum, was almost absent in the ileum and was most marked in the colon.

WATER AND ELECTROLYTE BALANCE

The water and electrolyte balance of all poisoned animals was severely affected. Inspection of the results shown in table 1 reveals a striking difference in the water intake and fluid output of experimental and control animals. Whereas all animals were consistent in their water intake during the preliminary 24-hour period, the intake of the experimental animals increased markedly in response to the administration of the toxic agents. A parallel change was observed in the fluid output which was 10-fold greater in some of the experimental animals than in the controls. Reference to the performance of *dogs 9, 10, 11, 12* and *13*, for which daily fluid collections are

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TABLE 1. WATER BALANCE AND EXCRETION OF ELECTROLYTE,
PHOSPHORUS AND NITROGEN

Dogs Poisoned at Time 0

GROUP	DOG	PERIOD	BALANCE OF WATER		OUTPUT OF SOLUTES				
			Intake	Output	Na	K	Cl	P	N
		days	*l*	*l.*	*mEq.*	*mEq.*	*mEq.*	*mM.*	*gm.*
Control	*1*	−1 to 1	0.65	0.25	8.0	7.5	6.4	10.0	5.25
		1 to 3	0.25						
	2	−1 to 3	0.67	0.19	2.2	13.6	3.5	16.8	8.56
	3	−1 to 0	0.0	0.16	14.0	9.9	12.6	7.4	2.99
		0 to 3	0.98	1.04	22.3	37.3	33.4	31.3	10.9
	4	−1 to 0	0.27	0.14	1.3	9.8	2.6	7.9	3.41
		0 to 3	0.54	0.34	3.0	30.8	3.6	20.6	9.83
Series I	*5*	−1 to 2	1.66	1.53	90.2	75.7	121.2	52.6	17.9
	6	−1 to 3	3.78	2.49	58.4	118.6	97.3	75.5	32.7
	7	−1 to 0	0.19	0.06	3.4	8.0	0.6	6.1	2.41
		0 to 3	1.51	1.16	43.1	83.1	93.3	59.8	19.0
	8	−1 to 0	0.37	0.08	1.9	9.6	3.5	5.7	3.75
		0 to 3	0.93	0.49	7.3	46.3	12.7	41.5	19.0
	9	−1 to 0	0.26	0.11	2.3	6.8	6.1	9.5	3.70
		0 to 1	1.30	0.61	15.2	5.8	21.4	0.3	0.16
		1 to 2	0.91	1.10	26.1	74.9	29.6	56.5	20.3
		2 to 3	0.47	1.20	20.1	59.4	16.4	41.2	11.7
		3 to 4	0.29	0.95	2.9	44.2	29.2	19.2	6.65
	10	−1 to 0	0.25	0.12	0.7	12.8	0.4	11.0	4.42
		0 to 1	1.30	0.90	19.6	59.6	26.6	39.8	9.00
		1 to 2	1.09	0.95	26.4	56.0	31.8	37.7	12.6
		2 to 3	0.70	1.20	2.9	64.4	58.2	39.2	12.6
		3 to 3+	0.32	0.45	13.6	21.2	25.9	10.4	3.34
	11	−1 to 0	0.0	0.10	18.9	12.2	13.0	11.5	3.85
		0 to 1	0.51	0.40	23.3	4.4	32.2	0.4	0.21
		1 to 2	0.90	0.76	24.0	55.9	28.2	40.3	14.1
		2 to 3	0.59	0.92	17.2	54.8	27.6	34.2	9.78
		3 to 3+	0.0	0.80	21.9	37.2	55.5	19.5	7.63
Series IIA	*12*	−1 to 0	0.20	0.09	3.0	7.9	0.4	9.8	4.16
		0 to 1	1.38	2.00	94.6	51.6	168.6	24.6	5.87
		1 to 2	1.25	0.50	14.5	25.0	19.5	9.5	1.51
	13	−1 to 0	0.03	0.10	9.8	11.4	11.9	10.5	3.61
		0 to 1	0.76	0.63	30.0	27.0	45.8	22.7	4.92
		1 to 2	1.31	1.60	87.0	66.3	129.3	38.2	9.59
		2 to 3	0.62	1.04	33.5	51.2	52.2	29.7	11.2
		3 to 4	0.40	0.60	19.8	37.3	29.9	20.6	8.79

TABLE 1—*Continued*

GROUP	DOG	PERIOD	BALANCE OF WATER		OUTPUT OF SOLUTES				
			Intake	Output	Na	K	Cl	P	N
		days	*l.*	*l.*	*mEq.*	*mEq.*	*mEq.*	*mM.*	*gm*
	14	−1 to 0	0.09	0.10	trace	6.4	0.2	7.8	3.19
		0 to 2	0.60	0.94	55.9	36.8	74.2	22.8	7.12
		2 to 2+	0.0	0.28	22.5	8.7	37.3	5.4	1.65
	15	−1 to 0	0.04	0.11	19.9	14.0	11.8	11.0	4.69
		0 to 2	0.31	0.32	10.4	28.7	20.7	22.8	7.44
		2 to 4	1.56	1.67	28.5	18.7	50.9	27.5	13.2
		4 to 8	1.24	1.43	trace	79.3	14.0	69.8	31.5
Series IIB	*16*	−1 to 0	0.03	0.13	5.7	14.9	1.1	11.7	5.44
		0 to 1	1.44	1.12	61.3	54.0	62.2	38.2	9.32
		1 to 2	0.73	0.70	24.9	39.0	41.0	39.0	12.0
		2 to 3	0.67	0.78	22.1	49.4	29.4	40.8	16.7
	17	−1 to 0	0.17	0.09	8.3	8.1	0.4	7.2	3.73
		0 to 2	0.92	0.71	31.6	49.9	50.7	32.8	9.22
		2 to 3+	0.21	0.63	34.8	26.8	40.3	20.3	8.47
	18	−1 to 0	0.38	0.37	trace	16.2	1.5	10.3	4.6
		0 to 2	0.80	0.52	24.7	56.2	33.0	51.7	20.0
		2 to 4	0.09	0.21	trace	46.3	17.6	20.6	8.41
		4 to 8	0.79	0.34	trace	32.9	8.3	32.3	17.0

shown in table 1, indicates that a marked increase in water exchange was evident within 24 hours. Indeed, it was not unusual to note that when the muzzles were removed from experimental animals of Series I, three to four hours after poisoning, the first act of the dogs was to drink avidly. A knowledge of the renal output during this period would be of great interest. Until this is ascertained, it cannot be stated whether the polydipsia or polyuria was primary.

Inspection of tables 1 and 3 reveals that the excretion of sodium and chloride was much greater in experimental than in control dogs, indicating a marked loss of extracellular electrolyte. The animals of Series IIA and IIB sustained a greater loss than the animals of Series I. This appears to be due to the more extensive vomiting and diarrhea exhibited by the former animals. Reference to table 1 shows further that the loss of extracellular ions was apparent within 24 hours after administration of nitrogen mustard.

Although as mentioned previously vomitus contributed to all the daily collections of Series IIA and IIB only a small part of the electrolyte loss of Series I can be accounted for by emesis. In several of the dogs of Series I the volume of vomitus was negligible as compared with the total volume of the fluid collection. In almost every instance in this group vomiting had ceased by the second day whereas the excretion of sodium and chloride persisted. Lastly in *dogs 9* and *11* there was complete separation in the collection of urine and vomitus due to voluntary retention of urine on the part

of the dogs during the first 24 hours of the experimental period.   A comparison of the electrolyte excretion of the first day with that of the second day indicates the relative contribution to the electrolyte loss by the gastric and renal routes respectively.   The extrarenal origin of the first 24-hour collection of *dogs 9* and *11* is also evident in the low values for phosphorus and nitrogen.

The extent to which diarrhea contributed to the loss of extracellular electrolyte was also greater in Series IIA and IIB than in Series I.   In Series I the incidence of diarrhea was variable and in no instance were watery stools evident during the first 48 hours.   Thereafter, diarrheal fluid contaminated the collections of *dogs 7, 9, 10* and *11* to a variable degree.   Inspection of the amounts of sodium and potassium in the excretions after the onset of diarrhea in these animals yields the information that diarrheal fluid contributed a minor portion of the total output.   *Dogs 5, 6* and *8* failed to evidence diarrhea during the course of the experiment.

Results of the calculation of changes in the volume of extracellular fluid appear in table 2.   The extent to which the extracellular fluid volume was altered as the result of the administration of amine mustard reflects to some extent the balance between water intake and fluid output.   Thus *dog 6* maintained extracellular fluid volume by ingestion of water.   In animals which drank less the volume of extracellular fluid diminished, i.e. in the case of *dogs 5, 9* and *13*.   Other animals exhibited an intermediate course.   Obviously in those cases in which extracellular volume was largely maintained in the face of the loss of extracellular electrolyte, the concentration of extracellular electrolyte was markedly decreased.

In table 3 it may be seen that the intoxicated animals with certain exceptions lost 10 or more per cent of initial extracellular sodium and even larger portions of initial extracellular chloride through the kidneys and gastrointestinal tract.   This is in marked distinction to the control animals and to *dogs 15* and *18* which survived as well as *dog 8* which for reasons explained above may have absorbed a smaller amount of amine mustard.   A study of table 3 reveals further that the extent of excretion of sodium and chloride was greater in animals of Series IIA and IIB than in those of Series I.   It is evident, therefore, that the systemic actions of methyl-beta-chloro-ethyl-ethylenimonium caused a more severe loss of extracellular electrolyte than resulted from fatal intoxication with *tris*(beta-chloroethyl)amine.   This appears to be associated with the extensive vomiting and diarrhea which was exhibited by the animals receiving the chlor-imine.   It is also important to observe that fatal intoxication with LD$_{50}$ doses of the chlor-imine in *dogs 14* and *17* resulted in losses of sodium and chloride similar in degree to those caused either by higher dosages of the same agent or by lethal amounts of *tris*(beta-chloroethyl)amine.   On the other hand, *dogs 15* and *18*, which survived after receiving LD$_{50}$ doses of the chlor-imine, showed only moderate though definite increases in electrolyte excretion.

Balance of Intracellular Sodium.   The excretion of chloride exceeded that of sodium by significant amounts in all poisoned dogs with the possible exception of *8* (table 3).   During the first two days of intoxication the small excess could be attributed to the amount of vomitus in the fluid excretions.   During the terminal portion of the observation periods, however, it was not uncommon for chloride to be excreted far in excess of sodium.   This is evident in table 1 where, in the cases of *dogs*

TABLE 2. WEIGHT AND ANALYSES OF SERUM AND BLOOD IN FASTING DOGS INTOXICATED WITH NITROGEN MUSTARD AND IN THEIR CONTROLS. CALCULATION OF EXTRACELLULAR FLUID VOLUME

Dogs Poisoned at Time o

GROUP	DOG	TIME	WEIGHT	Na	K	Cl	Total CO₂	H₂O	NPN	PLASMA OR SERUM PROTEIN	ECl
		day	kg.	mEq/l.	mEq/l.	mEq/l.	mM/l.	gm/100 gm.	mg/100 cc.	gm/100 cc.	
Control	1	−1	13.2	154	6.0	114					
		3	12.5	154	5.5	115					
	2	−1	9.8	155	5.6	119				6.3	2.45
		3	9.0	149	6.3	116				5.4	2.49
	3	0	23.56	141		106	25.1	92.3	17.0	6.0	5.88
		3	22.82	135		105	25.4	93.2	24.2	5.7	5.76
	4	0	11.21	136		105	23.3	91.6	25.0	7.2	2.80
		3	10.94	136		100	22.4	92.4	28.8	6.9	2.91
Series I	5	−1	16.1	141	5.1	108				6.4	4.02
		2	14.2	145	5.4	94.5				8.3	3.44
	6	−1	13.6	144	4.8	108				6.4	3.40
		3	12.5	127	5.8	82.5				6.7	3.41
	7	0	11.42	140		106	22.6	92.3	28.2	6.1	2.86
		3	10.06	134		84.5	33.9	91.8	49.6	5.4	2.61
	8	0	14.17	141		106	24.0	92.5	49.7	6.1	3.54
		3	13.85	137		101	24.6	92.7	31.4	6.1	3.59
	9	0	17.21	147	4.0	114	21.7	91.6	21.7	7.3	4.30
		2	16.33	137		102	20.5	91.3		7.2	4.35
		3	15.37	142	5.1	103	23.2	90.7	52.2	8.3	4.13
		4	14.31	149	4.4	111	21.7	90.2	107	8.7	3.58
	10	0	14.29	143	4.6	111	20.6	92.3	22.5	6.9	3.57
		2	13.61	132	4.1	98.9	20.8	92.2	32.3	6.7	3.47
		3	12.87	131	4.1	92.6	23.5	91.8	48.3	7.3	3.15
	11	0	17.72	145	4.5	108	22.3	91.3	22.6	7.1	4.43
		2	16.95	139	3.8	100	24.1	91.3		7.5	4.26
		3	16.19	140	4.4	96.3	24.7	91.3	37.9	8.1	4.15
Series IIA	12	0	12.10	146		108	23.1	92.8	32.0	5.5	3.13
		2	11.00	134		61.6	33.7	91.0	177	6.9	2.70
	13	0	13.19	146		111	20.4	91.8	29.5	6.3	3.30
		2	12.22	135		82.8	33.3	90.9	36.2	7.2	2.54

TABLE 2—*Continued*

GROUP	DOG	TIME	WEIGHT	CONCENTRATION IN SERUM						PLASMA OR SERUM PROTEIN	ECl
				Na	K	Cl	Total CO₂	H₂O	NPN		
		day	kg.	mEq/l.	mEq/l.	mEq/l.	mM/l.	gm/100 gm.	mg/100 cc.	gm/100 cc.	l.
		3	11.68	129		67.9	41.2	91.1	36.0	7.4	2.44
		4	10.98	115		66.1	39.4	91.6	21.1	7.1	2.12
	14	0	11.1	144		104	24.3	92.7	33.5	5.7	2.78
		2	10.0	138		92.1	30.0	91.6	40.5	6.7	2.40
	15	0	12.7	150		110	25.2	92.3	23.5	6.2	3.18
		2	11.9	139		104	25.7	91.9	27.6	6.6	3.16
		4	11.2	135		88.1	33.2	91.9	44.6	6.8	3.22
		8	10.3	137		87.5	31.8	92.7	39.5	6.1	3.13
Series IIB	*16*	0	14.68	152		111	23.1	92.3	32.1	5.9	3.67
		2	13.38	135		90.2	24.7	91.8	71.7	6.8	3.49
		3	13.27	129		83.2	27.0	92.2	69.5	6.3	3.49
	17	0	8.6	141		106	23.6	93.3	30.8	5.2	2.15
		2	8.2	135		82.1	31.3	91.9	114	5.9	2.19
	18	0	13.3	144		113	20.7	93.2	37.7	5.3	3.32
		2	13.0	141		106	22.4	92.9	34.0	5.5	3.24
		4	12.4	140		106	22.7	93.0	30.8	5.5	3.08
		8	11.3	139		107	23.6	93.0	32.4	5.6	2.99

9 and *10*, the extreme condition was observed in which the excretions were almost sodium-free and yet contained large amounts of chloride. Nevertheless, the fact that chloride was excreted in excess was not uniformly reflected in all dogs by a significant change in total serum CO₂ (see *dog 9*, table 2). This may indicate a passage of sodium into cells in exchange for potassium. Indeed, calculation reveals gains of intracellular sodium in many of the experimental animals following intoxication as well as a direct relationship between increases of total serum CO₂ and of the calculated value, $(b_{Na} - b_{Cl}) - b'_{Na}$ (table 3). The latter value may be taken to represent the calculated maximal increase in the available base of extracellular fluid expected to result from the excretion of chloride in excess of sodium. The only notable exception to the apparent correlation are the results from *dog 13* between zero and four days after intoxication. Even in this animal the correlation fails only after the third day of intoxication. Inspection of the values for b'_K in table 3 also reveals that most of the experimental dogs lost sufficient potassium from cells, beyond those amounts resulting from catabolism, to account for the instances in which significant gains in intracellular sodium were calculated.

Potassium, Phosphorus and Nitrogen Excretion. Intoxicated animals excreted potassium, phosphorus and nitrogen greatly in excess of controls (table 1). In sev-

eral instances such as *dogs 10, 12, 13* and *16* daily collections of excreta revealed that the enhanced output of potassium, phosphorus and nitrogen was evident within 24 hours after intoxication. Voluntary retention of urine on the part of *dogs 9* and *11* undoubtedly accounts for the low values obtained analytically in the collections of the first 24 hours.

The increased excretion of intracellular cation in experimental animals can in part be attributed to an increased cellular catabolism. This might be expected to

TABLE 3. COMPARISON OF THE BALANCE OF INTRACELLULAR SODIUM WITH THE EXCRETION OF CHLORIDE IN EXCESS OF SODIUM, WITH CHANGES IN SERUM CO₂, AND WITH EXCRETION OF POTASSIUM IN EXCESS OF PROTEIN CATABOLIZED. TOTAL ELECTROLYTE LOSSES IN EXPERIMENTAL AND CONTROL DOGS

SERIES	DOG	PERIOD	$b_{Na}-b_{Cl}$	b'_{Na}	$(b_{Nb}-b_{Cl})-b'_{Na}$	CHANGE IN TOTAL SERUM CO₂	b'		PORTION OF INITIAL EXTRACELLULAR ELECTROLYTE LOST	
									Na	Cl
		days	*mEq.*	*mEq.*	*mEq.*	*mM/l.*	*mEq.*	*mEq/kg/day*	%	%
I	5	−1 to 2	+31	−17	+48		−22	−0.48	15	25
	6	−1 to 3	+39	−1	+40		−21	−0.40	11	23
	7	0 to 3	+50	+8	+42	+11.3	−22	−0.68	10	27
	8	0 to 3	+5	0	+5	+0.6	+5	+0.12	1.4	3.1
	9	0 to 4	+32	+32	0	0.0	−42	−0.65	10	17
	10	0 to 3	+68	+51	+17	+2.9	−70	−1.72	12[1]	32[1]
	11	0 to 3	+24	+4	+20	+2.4	−30	−0.59	13[1]	26[1]
II A and B	16	0 to 3	+24	+5	+19	+3.9	−19	−0.46	19	29
	12	0 to 3	+79	−18	+97	+10.6	−22	−0.95	23	50
	13	0 to 3	+77	+22	+55	+20.8	−67			
		0 to 4	+87	+80	+9	+19.0	−82	−1.69	34	62
	14	0 to 2	+18	+10	+8	+5.7	−15	−0.71	19[2]	34[2]
	15	0 to 4	+33	+4	+29	+8.0	+15	+0.31	7.9	18
	17	0 to 2	+19	−29	+48	+7.7	−8	−0.48	21[1]	31[1]
	18	0 to 4	+26	+24	−2	+2.0	−20	−0.39	5.1	12
Control	2	−1 to 3						+0.32	0.5	1.2
	3	0 to 3						−0.02	2.5	4.7
	4	0 to 3						−0.02	0.8	1.2

[1] Calculated for 0 to 3 + days.   [2] Calculated for 0 to 2 + days.

be the result of the known cytotoxic actions of amine mustards and is evidenced by the enhanced excretion of nitrogen and phosphorus in experimental dogs. However, an analysis of the amount of potassium in the fluid excretions in relation to the amount of nitrogen reveals that the experimental animals excreted intracellular cation in excess of those quantities which could be accounted for solely on the basis of enhanced cellular catabolism.

Values for potassium lost as the result of processes other than catabolism are shown in table 3. The b'_K values shown in table 3 for experimental animals may be compared with the values derived from 17 periods of collection from animals prior to intoxication and from controls which ranged between −0.18 and ±0.36 mEq/kg/day

and averaged ±0.08. It is evident that in all experimental animals with the exception of *dogs 8* and *15* the losses of potassium during periods following intoxication were excessive.

In view of the losses of excess potassium suffered by most of the experimental dogs it is worthwhile to consider briefly the exceptional dogs, *8* and *15*. For reasons outlined previously the former animal probably received a sub-lethal dose of nitrogen mustard; the latter animal survived the period of intoxication. Both animals sustained no significant losses of potassium in excess of that presumed to result from cellular catabolism. It is, therefore, of interest to note that *dog 8* likewise showed no significant increase over the control period in water intake, fluid output and sodium and chloride excretion. Moreover, *dog 15* exhibited changes in these excretory processes to a lesser degree than did experimental animals which failed to survive. The moderate increase in excretion of potassium and nitrogen found in *dogs 8* and *15* can be attributed to a moderate dissolution of lymphoid and myeloid tissue. It may finally be noted that *dog 18*, a surviving animal like *15*, exhibited losses of excess potassium which were intermediate between those of controls and those of non-survivors.

Changes in Plasma Protein. It will be noted in the data of table 2 that a reciprocal relationship exists between extracellular fluid volume and plasma protein concentration (the only notable exception is *dog 7*). This is particularly evident in the response of the plasma protein concentration of *dog 9* in that during the first 48 hours when extracellular fluid gained 0.08 liters plasma protein fell slightly. During the next 48 hours the decline in extracellular fluid volume was associated with a marked increase in plasma protein concentration. The reciprocal relationship between plasma protein concentration and the volume of the extracellular fluid suggests that the impermeability of the capillary wall to protein remained unimpaired.

DISCUSSION

The above data present evidence for *a)* loss of extracellular electrolyte, *b)* loss of intracellular electrolyte and *c)* increased catabolism as the result of fatal intoxication with nitrogen mustard. However, the rôle of electrolyte and water imbalances in the sequence of events involved in the fatal syndrome is not clear. To be sure, there is ample evidence justifying the conclusion that death is associated with a marked inability to retain extracellular electrolyte. Regardless of the mechanism, whether through emesis, diarrhea or failure of renal reabsorption, the loss during the first three to four days following intoxication of 10 or more per cent of total extracellular sodium and 17 or more per cent of total extracellular chloride is associated with a fatal outcome. Moreover, animals which survive LD_{50} doses show a transient loss of sodium and chloride which appears significantly increased over that of starving controls but which fails to assume the proportions of the electrolyte disturbances associated with death. Nevertheless, the extent of the loss of extracellular electrolyte by itself seems insufficient to explain the fatal outcome of intoxication in all animals. In experimental animals losses of one third to one half of total extracellular electrolyte are usually considered fatal (20). Of the present animals none of Series I and only a few individuals of Series IIA and IIB sustained losses to this extent. Moreover, it is difficult to attribute death solely to a loss of extracellular electrolyte when in an

extensive series of experiments Smith and co-workers have shown that supportive therapy designed to maintain extracellular electrolyte and fluid intact, benefits but fails to save a significant number of animals from the systemic effects of nitrogen mustards (6).

It is of interest to consider the mechanism by which extracellular electrolyte is lost as the result of nitrogen mustard poisoning. A portion was lost as the result of vomiting; another fraction was lost later in the course of poisoning from the intestinal tract due to diarrhea. However, in the animals of Series I which received *tris*(beta-chloroethyl)amine the greatest amount appeared to have been excreted by the kidneys. This must be attributed to a renal insufficiency in the reabsorption of sodium and chloride. The wastage of extracellular electrolyte exhibited by the poisoned dogs of Series I is not unlike that encountered in adrenal insufficiency. However, there was no evidence of any rise in serum potassium despite the fact that large amounts of this cation were made available for excretion during the experimental period. Moreover, no pathological change in the adrenal has been observed to support such a thesis. One is left with the conclusion that *tris*(beta-chloroethyl)amine produces a functional disturbance of the kidney of such magnitude as to interfere with homeostasis. Presumably the animals of Series II which received methyl-beta-chloroethyl-ethylenimonium were similarly affected although for reasons advanced previously renal losses could not be distinguished readily in this group from losses due to vomiting and diarrhea.

Alteration of the volume of the intracellular fluid likewise seems insufficient to account for the morbid changes which occur during fatal intoxication. Approximate values for changes of cell fluid volume may be estimated as the difference between the observed changes in weight and the calculated changes in the volume of extracellular fluid during the period of intoxication. Such calculations reveal depletions of cell fluid in all of the animals which succumbed with the exception of *dog 9* of not more than 10 per cent of initial body weight. *Dog 9* lost cell fluid to the extent of about 13 per cent of initial body weight, a result which is probably associated with the fact that this animal exhibited the most negative water balance. These values appear to be well within the limits compatible with viability in dogs which have been subjected to chronic deprivation of food and water (21). Nevertheless, the values for cell fluid loss in the present animals were significantly higher in poisoned dogs than in their starving controls.

Lastly the contribution of the loss of intracellular potassium to the lethal action of the mustards should be considered. As already stated this can only partly be accounted for on the basis of increased catabolism. It is important to examine the possibility that the loss of potassium in excess of protein catabolized can be associated with the cellular dehydration noted in poisoned animals. Elkinton and co-workers (17, 21, 22, 23) have shown that significant loss of excess potassium is obtained in dogs subjected to procedures which lead to depletion of cell water. The depletion may be instigated diversely by chronic deprivation of food and water, by parenteral administration of hypertonic solutions of NaCl, by removal of fluid from the peritoneal cavity after intraperitoneal administration of hypertonic solutions of NaCl or glucose, or by urea diuresis. All of the above procedures cause a primary loss of extracellular fluid or an increase in concentration of extracellular electrolyte. However, in the

present animals loss of cell water occurred usually in the face of a loss of extracellular electrolyte and a reduction in the osmotic pressure of extracellular fluid. In view of these facts it is not surprising that inspection of tables 1, 2 and 3 reveals no correlation between the balance of excess potassium and either the balance of water or the extent of change of intracellular fluid as estimated from changes in weight and E_{cl}. Indeed, *dog 6* showing the most positive water balance and a moderate change of body weight and fluid volume exhibited a negative balance of excess potassium (calculated in mEq/kg/day) which differed little from that of *dog 9* showing the most negative water balance and the greatest loss of cell fluid.

Another possible explanation for the loss of intracellular cation is suggested by the recent clinical and experimental observations that drastic changes in the composition of the extracellular environment either induced experimentally or resulting from excessive therapy with sodium chloride or from severe diarrhea may often be accompanied by unexplained losses in intracellular potassium (20, 24). The deficits of intracellular potassium are often associated with abnormally high concentrations of intracellular sodium and serum bicarbonate. However, in the present experimental animals which consistently underwent severe changes in the composition of the extracellular fluid due to emesis, diarrhea and renal impairment of electrolyte reabsorption, the loss of potassium cannot be correlated with the extent of the loss of extracellular electrolyte or change in the bicarbonate concentration of serum (Series I versus Series II, table 3). Furthermore, in the above experiments excretion of potassium in excess of nitrogen occurred as early as the first experimental day at a time when distortion of the extracellular fluid had not yet become marked.

It is tempting to attribute the loss of intracellular potassium to a direct cytotoxic action of nitrogen mustard which affects metabolic activity so that the cell is unable to maintain the integrity of its cationic structure. This type of action would presumably result in the exchange of sodium and potassium across the cell membrane. Potassium in an extracellular site would be preferentially excreted by the renal tubule with an equivalent of anion. The close correlation between b'_K and $b_{Na} - b_{cl}$ (table 3) is in keeping with such a sequence of events. Although the correlation between b'_K and b'_{Na} is less obvious, nevertheless, significant increases in intracellular sodium are observed in the dogs which show the greatest loss of excess potassium.

That an intracellular potassium loss would contribute further to the cytotoxic action of nitrogen mustard appears likely. The observations of Darrow on the importance of replacing potassium loss sustained in diarrhea are pertinent in this regard (20). Thus a cytotoxic agent could presumably initiate a vicious cycle of events in which the biochemical lesion initially produced by a toxic agent could be sustained by progressive depletion of potassium until ultimate functional failure of the cell. In the case of nitrogen mustard poisoning there may be the further contribution of distortion in the chemistry of the extracellular fluids secondary to impaired renal function, diarrhea and vomiting. Although no single mechanism would be sufficient to cause death, the combination presumably results in conditions incompatible with survival.

SUMMARY

Systemic intoxication by *tris*(beta-chloroethyl)amine and methyl-beta-chloroethyl-ethylenimonium in dogs is characterized by an extensive loss of extracellular and

intracellular electrolyte and a greatly increased fluid intake and output. The loss of extracellular electrolyte may be due in part to vomiting and diarrhea but also results from a renal defect in the reabsorption of sodium and chloride. A large portion of the intracellular electrolyte lost can be accounted for on the basis of increased catabolism, presumably for the most part of lymphoid and myeloid tissues. The remainder of the potassium excreted is in excess of protein catabolized. The extensive loss of electrolyte exhibited by animals which receive lethal doses appears to be an essential component of the fatal syndrome. However, it is not possible to conclude that the deficits of both extracellular and intracellular electrolyte are the direct cause of death.

The loss of potassium in excess of the amounts appearing as the result of enhanced catabolism may be associated with a unique cytotoxic action of nitrogen mustards. However, other possible causes for the unexplained excesses in potassium excretion are discussed.

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WATER INGESTION AND EXCRETION IN RATS UNDER SOME CHEMICAL INFLUENCES[1]

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TWO different concepts prevail concerning the regulation of water turnover in mammals. One is that intake is a haphazard process and that accurate corrections of water content are performed by excretion. The other is that ingestion, while intermittent, is as sensitive to modifications of body water content as is excretion. The following experiments were designed to test some modifications that could be elicited in water intake and in water output. The ulterior object of the investigation was to elucidate some of the factors in thirst, the urge to drink. Factors in water intake and output were to be studied separately and together.

Experiments, conducted upon male rats whose mean body weight was 220 gm., were of three sorts. In Series I, rats were furnished diluted milk (2.6% solids) so that large volumes were ingested by them (1). The amounts of ingesta (from tube-tipped graduated cylinders) and of collectable urine were measured in hourly periods after intramuscular injection of a chemical agent. In this manner certain agents that inhibit water turnover were identified. In order to find (Series II) whether ingestion alone was influenced by the agents, rats kept on a diet of dried whole milk were without drinking water for 48 hours and were injected with agents shortly before drinking of distilled water was again allowed. In order to ascertain (Series III) whether water excretion itself was inhibited, rats kept on dried milk up to the hour of experiment were given by stomach tube six doses of 5 per cent of the body weight of water, at 30-minute intervals. Urine was collected but no drinking water was available during this test.

Series I. Rats that were furnished 2.6 per cent milk solids in distilled water as the only source of intake ingested approximately their own body weights of liquid every day. The mean fluid intake and urinary output were quite uniform hour
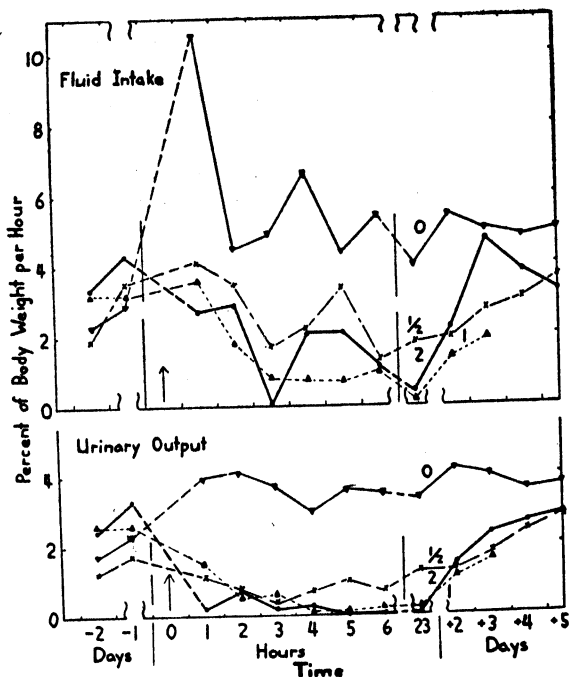
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Fig. 1. INTAKE OF DILUTED MILK AND OUTPUT OF URINE by rats injected with pitressin in oil at zero time. Each line represents the mean observed in 7 tests. The first 2 and last 4 points of each line represent daily averages; the 23-hr. point is the mean of 17 hr.; the intermediate points are for single hr. Pitressin tannate in oil was injected intramuscularly at the arrow in doses of 0, $\frac{1}{2}$, 1 or 2 U/rat. The excessive intake in the first hr. followed the unavailability of milk mixture for about one hr. before injection. All the doses ($\frac{1}{2}$, 1 or 2 U.) evidently inhibited both intake and output for more than 24 hr.

Fig. 2. INTAKE OF DILUTED MILK by rats injected with pilocarpine at 2 hr. Each line represents the mean result of 4 tests. The first 2 and last 2 points represent daily av.; the 23-hr. point is the mean of 16 hr.; all other points are single hr. Pilocarpine greatly suppressed the fluid intake at first, but the 4 mg. injection greatly enhanced the intake in several later hours.
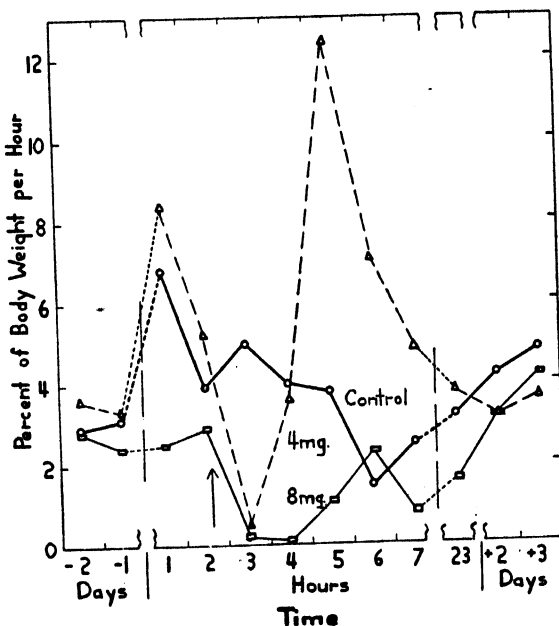
after hour (fig. 1). Both were temporarily suppressed by administration of sufficient atropine, pilocarpine (fig. 2) and postpituitary extracts (fig. 1). Other substances tested, namely acetylcholine and epinephrine, had no significant effect (table 1).

It is widely recognized that pilocarpine and atropine antagonize one another in many of their physiological effects.   When the two were administered successively to the same rats (table 1), water turnover was in most instances reduced.   Since

TABLE 1. INFLUENCES OF SEVERAL AGENTS UPON WATER EXCHANGES
(Means and Standard Errors)
Series I

AGENT	DOSE/RAT (INTRAMUSCULARLY)	NO. OF TESTS	OUTPUT OF URINE % wt/hr. in first 24 hrs.	INTAKE OF 2.6% MILK % wt/hr. in first 24 hrs.	INTAKE IN SECOND HOUR % wt.	INTAKE % wt/hr. in first 5 hrs.
Control		15	3.1 ±0.43	4.2 ±0.48	5.5 ±1.11	5.2 ±0.38
Acetyl-choline	300 μg. 600 μg.	4	2.2	3.0	2.2	2.3
Doryl	50 μg. 200 μg. 600 μg.					
Atropine	17 μg. 50 μg.	4 4	2.3 2.2 ±0.4	3.2 2.9 ±0.4	2.2 ±0.4	2.4 2.0 ±0.5
Atropine, then Pilo-carpine	17 μg. + 0.5 mg. 50 μg. + 4.0 mg. 100 μg. + 4.0 mg.	2 2	3.4 2.2	4.5 2.9	3.4 2.2	1.0
Epinephrine in oil	300 μg.	3	2.9	3.9	2.9	3.4
Pilocarpine	0.5 mg. 1.0 mg. 2.0 mg. 4.0 mg. 8.0 mg.	2 4 4	3.0 3.2 0.96	3.9 4.8 (fig. 2) 1.5 (fig. 2)	3.0 3.2 0.96	3.7 5.7 0.9
Pitressin in oil	0.5 U. 1.0 U. 2.0 U.	7 7 7	1.2 ±0.06 0.26 ±0.32 0.08 ±0.14	2.1 ±0.17 0.53 ±0.08 0.56 ±0.06	1.2 ±0.06 0.26 ±0.32 0.08 ±0.14	3.0 ±0.86 1.5 ±0.15 1.3 ±0.26
Pituitrin aqueous	1 to 3	22	2.5 ±0.68	3.0 ±0.77	2.5 ±0.68	3.4 ±0.68

each of the two agents alone suppressed the turnover anyway, it would be unlikely that when combined they would antagonize one another in this phenomenon.

Dilution of food, therefore, inducing a continuous voluntary turnover of large quantities of water, furnished a basic pattern of rapid water exchange.   Three chemical preparations in appropriate doses were capable of suppressing part of the excessive turnover.   The question then remained as to whether the agents acted upon some

identifiable part of the water exchange; it was partially answered in Series II and III below.

   Series II. Rats that had been without water (but with dry food) for 48 hours lost about 9 per cent of the body weight. When then allowed to drink they ordinarily drank enough water in one hour to make up about three fourths of the weight that had been lost (fig. 3), but when certain doses of pilocarpine were given, intake was severely inhibited. The inhibition did not last through a whole day, however, and recovery from water deficit was largely achieved before food was again furnished to the rats.
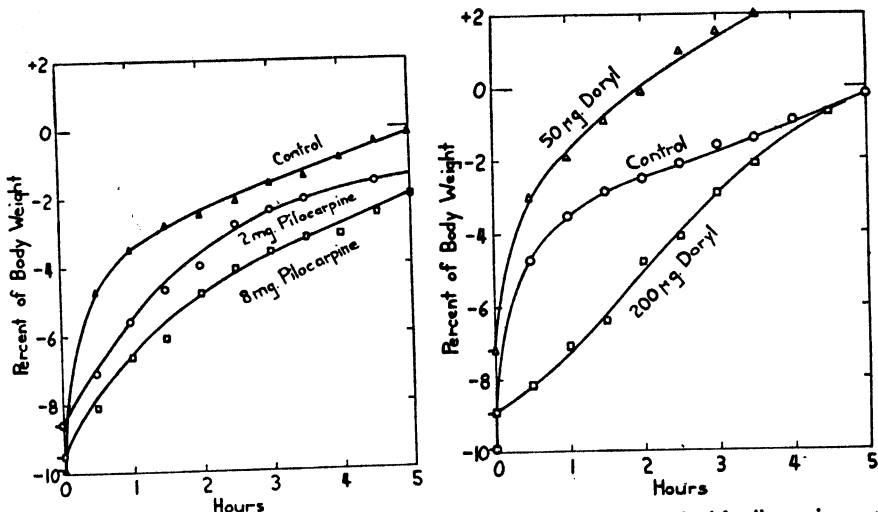

Fig. 3 (*left*). CUMULATIVE INTAKES of distilled water by rats injected with pilocarpine 0.5 hr. before water was first allowed at zero time. Rats had been deprived of water but allowed dry food (whole milk powder) for 48 hr. previously. Body wt. 48 hr. ago was taken as zero. Each point is the mean of 29 (o dose), 9 (2 mg.) and 7 (8 mg.) tests. Drinking was inhibited in the first half hour by both doses.

Fig. 4 (*right*). CUMULATIVE INTAKES of distilled water by rats that had been deprived of water during the previous 48 hr. They were allowed food during that period, but not after zero time. The body wt. 48 hr. previously was taken as zero wt. Controls, 29 tests; 50 μg. doryl, 4 tests; 200 μg. doryl, 6 tests, animals prostrated for 2 hr., which accounts for their small initial intake. Doryl was injected into a leg muscle 0.5 hr. before zero time at which water was first allowed.

   Of the hormone preparations tested, only pilocarpine and doryl (carbaminoylcholine) had significant influences upon water ingestion (table 2). The manners of action of the two agents were markedly different. After pilocarpine the animals mouthed the drinking tubes but took very little from them. Often they withdrew to other parts of the cages, but soon approached the tubes again. This may be designated as a behavior of frustration. After doryl (fig. 4) the animals were prostrated, to the point where they were physically unable to drink, by a dose sufficient to inhibit drinking at all. Pilocarpine could be said to exert an effect upon drinking, but doryl an effect upon all neuromuscular activities including drinking. In this way it is demonstrated that of the three agents that reduced the rate of water turnover,

one of them (pilocarpine) influences water intake under conditions where intake is not inhibited by the accumulation of a water excess in the body.   In dehydrated rabbits a similar inhibition of drinking was reported by Pack (2).   In dehydrated men a lesser dose of pilocarpine had no influence upon drinking (3).   The effects of

TABLE 2.   INFLUENCES OF SEVERAL AGENTS UPON WATER EXCHANGES
(Means and Standard Errors)

AGENT	DOSE/RAT (INTRAMUSCULARLY)	SERIES II		SERIES III	
		NO. OF TESTS	INTAKE OF WATER AFTER 48 HR. WITHOUT WATER % wt. in first hr.	NO. OF TESTS	OUTPUT OF URINE AFTER 6 x 5% WT. IN 3 HRS. % wt. in 4th hr.
Control		29	6.4 ±0.38 (fig. 3)	25	6.0 ±0.25
Acetylcholine	300 μg. 600 μg.	4	6.1	4	6.4 ±0.6
Doryl	50 μg. 200 μg. 600 μg.	4 6 4	5.3 (fig. 4) 1.8 Prostrated Killed	5	4.9 ±0.4
Atropine	17 μg. 50 μg.	4	5.0	3 6	6.3 ±1.3 5.0 ±0.4 (fig. 3)
Atropine, then Pilocarpine	17 μg. + 0.5 mg. 50 μg. + 4.0 mg. 100 μg. + 4.0 mg.	4	5.3	2	4.1 ±2.1
Epinephrine in oil	300 μg.				
Pilocarpine	0.5 mg. 1.0 mg. 2.0 mg. 4.0 mg. 8.0 mg.	3 2 9 3 7	7.4 7.1 3.0 3.9 ±1.0 (fig. 3) 2.9 ±0.8 (fig. 3)	3 4 4	5.6 ±0.4 5.5 ±0.8 5.0 ±0.7
Pitressin in oil	0.5 U. 1.0 U. 2.0 U.	5	5.5		
Pituitrin aqueous	1 to 3 U.	4	4.5		

pilocarpine in all species are transient and show themselves only when observations are made in periods of an hour or less.

Other effects of pilocarpine and doryl were visible.   Both of them in all the doses tested (but none of the other agents) produced the well-known secretion of red pigment (protoporphyrin) in eyes and nose.   Pilocarpine in doses of 4 and 8 mg. (and

no other agents) produced diarrhea; this was not seen until one hour after drinking began. Both substances (and no other agent) produced drooling salivation. It has been supposed by other investigators that the presence of copious saliva in the pharynx is important in the inhibition of water drinking (e.g. Cannon, 4). We observed as much salivation after 50 μg. of doryl and after 0.5 mg. of pilocarpine, which did not inhibit drinking, as after larger doses of each that did suppress drinking. This fact seems to constitute clear evidence that water intake is not diminished merely by having the throat flooded with saliva.

Postpituitary agents have sometimes been supposed to modify the urge to drink. Physiologists familiar with the syndrome of diabetes insipidus suggest that these agents will generally reduce the amounts drunk. Those who think of pituitrin as
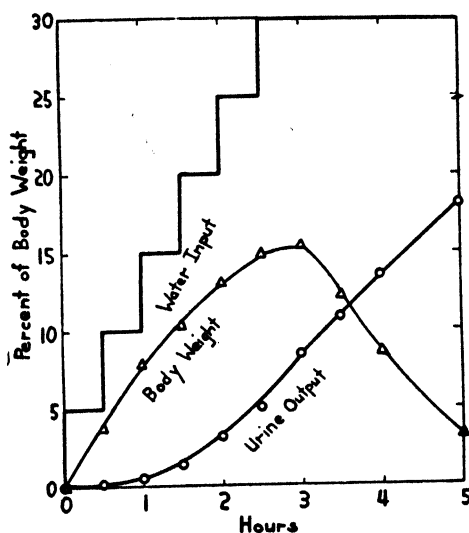

Fig. 5. CUMULATIVE EXCHANGES OF WATER and body wt. in rats to which water was forced by stomach tube in 6 portions. Each point is the av. of 6 tests; 50 μg. of atropine were injected 0.5 hr. before zero time. The substance injected produced no difference from controls.

antidiuretic are likely to suggest that drinking will be enhanced by it. The present experiments show that neither effect is significantly realized in normal rats.

Series III. The capacity to excrete water at high rates was tested by administering large volumes of water by stomach tube. Thirty per cent of the body weight of water was given within three hours. After the last dose the body weight was usually 13 per cent in excess and a rate of urine formation of 6 per cent of the body wt/hr. was regularly reached. When atropine was given, no significant modification occurred in the rate of urinary excretion even for an hour or two (fig. 5). Not all of the agents under consideration were tested here (table 2) because numerous tests of antidiuresis have been reported by other investigators.

The antidiuretic properties of postpituitary extracts are well known (5, 6), but it may be recalled that in some doses the extracts enhance urinary flow (7). Epinephrine has not been found to be antidiuretic in rats; in some doses it even accelerates diuresis (8). Acetylcholine is believed to oppose diuresis by exciting the postpituitary gland to secrete antidiuretic substance (9, 10); though after injection its influence is short-lived, perhaps due to its rapid inactivation.

The upshot of the investigation is that atropine, pilocarpine and postpituitary substances may temporarily reduce the turnover of excessive water by rats. Pilocarpine in large doses inhibits drinking, pituitrin inhibits excretion and atropine succeeds in affecting water turnover only in the second hour after it is injected. The results serve as a warning against too literal a partitioning of the diverse water exchanges.

<div align="center">COMMENT</div>

The tests here reported demonstrate clearly that rapid turnovers of water may be limited at intake as well as at output. Whenever intake of dilute food mixture is reduced (by pilocarpine), output is automatically reduced. Whenever output is reduced (by pituitrin), intake of dilute food is promptly reduced. In the latter case it appears probable that the urge toward ingestion is stopped by the incipient accumulation of water in the body. The impairment of either ingestion or excretion will modify water turnover. Or, turnover is not regulated wholly, if at all, at the organs of exchange, but is regulated more nearly in accordance with the water content of the body. Ingestion and excretion are means of correcting a deficit or an excess, when they are not thrown out of action by an agent such as one of the substances here studied.

At present there seems to exist no evidence that rats or any other mammals regulate water content by the repeated correction of deficits, any more frequently than they regulate it by the correction of excesses. Exchanges by ingestion and by excretion are equally dependent upon other factors; they are intimate parts of a complex.

The methods here employed serve as assay procedures for substances that affect water exchange. By the method of Series I it is possible to assay atropine, pilocarpine or postpituitary hormones without any manipulative administration of water (by tube or needle). By the method of Series II any modifier of drinking may be evaluated; such modifiers may be termed antiposic agents. By the particular procedure of Series III, antidiuretic agents may be assayed under conditions of maximal water excretion.

Theories of 'thirst' have been mainly concerned with the localization of an effective excitation to drink. No agent has been found that *enhances* water intake without the intervention of bodily dehydration. The urge to drink has been *suppressed* appreciably by pilocarpine and doryl. Both these agents are regarded as cholinergic. It is not yet demonstrated that cholinergic substances in general inhibit drinking. The points of action of such substances are so numerous that no profitable speculations can at present indicate their locations. Local administrations of such substances may eventually help to define their points of action in the urge to drink.

<div align="center">SUMMARY</div>

By diluting the food with large proportions of water, rats were induced to ingest large volumes of water which were continuously excreted. The large turnovers were inhibited by administering intramuscularly atropine, pilocarpine or postpituitary extracts. After rats had been without water for 48 hours, maximal ingestion was secured when water again became available. Ingestion and thirst were inhibited by

certain large doses of pilocarpine and of doryl. The inhibition was not found in all doses that stimulated excessive salivary flows. By administering water by stomach (6 doses, each 5% of the body wt.), water diuresis was induced. Of the agents tested, only postpituitary extracts appeared to be potent in suppressing the diuresis. Rapid turnovers of water in rats are regulated no more by output than by intake. Both exchanges are part of a complex which at present defies breakdown and which accords with the water content and (no doubt) other properties of the body.

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PARTIAL NEPHRECTOMY AND THE WATER EXCHANGES OF RATS[1]

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WHEN rats ingested large quantities of water in obtaining food with which it was mixed (1), large volumes of urine were also excreted. This dietary regime provided an easy method by which to pass excess of water through the animals and hence made feasible a study of the consequences of excessive turnover of water.

The present investigation is a partial exploration of some adaptive mechanisms of water exchanges. The question arose, would ingestion be diminished if the excreting tissue were reduced? Further, does the amount of renal tissue determine or modify water diuresis? It is well known that partial nephrectomy leads to hypertrophy of remaining renal tissue. Does high turnover of water also influence the rate or amount of hypertrophy? Answers to these questions are given here.

PROCEDURE

Each rat was maintained in a metabolism cage, in a cabinet kept at 27°C. In Series I the sole source of intake was a drinking cylinder containing 250 ml. of dilute milk. The milk mixture had 2.6 per cent of solids and was made each 24 hours by mixing 25 ml. of canned fortified milk (Formulac) with 0.5 gm. of benzoic acid and 225 ml. of distilled water. Of this liquid, rats drank quantities approximately equal to their own body weights each 24 hours (1).

Male rats of 150- to 200-gm. weight were given 3 to 10 days in which to become accustomed to the diet; then three days of control observations were allowed. On zero day rats were nephrectomized through flank incisions under ether anesthesia, in three diverse ways. In *Group A* a single kidney was excised (1 nephrectomized) after decapsulating it and tying the vessels of its hilus within the capsule. In *Group B* a half of one kidney was decapsulated and excised after tying a coarse thread through its middle; at the same time the other kidney was totally excised (1½ nephrectomized). In *Group C* half a kidney was excised alone, followed seven days later by excision of the remaining whole kidney (½ + 1 nephrectomized). In every case care was taken to leave intact the adrenals and their blood vessels. The period of the surgical operation deprived the rats of food and drink over several hours. Both the operated individuals of the three groups and the unoperated controls were returned to the same cages and diets as before. Subsequent intakes and urinary outputs were measured in 24-hour periods.

In Series II, male rats were tested, before and after 1½ nephrectomy, for excretory capacity. By forcing water into them through a stomach tube, the excretion of water attained a maximal rate, independently of the urges to drink. Warm distilled water equal to 5 per cent of the body weight was given at intervals of one-half hour for six or three administrations. Urine as spontaneously voided was collected in special small funnel-cages during at least six hours; its volume and the body weight were recorded every half hour. These animals were supplied *ad libitum* with dried whole milk and water.

At surgical operation the excised renal tissue was placed in a tared weighing bottle. After the tissue's wet weight had been ascertained, it was dried in an oven at 100°C. for two or three days until a minimal weight was attained and checked. With certain exceptions each rat was killed at 7 to 18 days after operation, if it survived that long, and its remaining kidney was then treated similarly. These determinations furnished information as to the extent of hypertrophy and its composition.

Daily Water Turnover (Series I). Rats subjected to loss of $1\frac{1}{2}$ kidneys at one operation (*Group B*) drank about one third of the usual amount of dilute food in the first 24-hour period (fig. 1). Correspondingly the output of urine was small. On subsequent days the intake gradually increased and, at the end of about seven days, stabilized at the rate of water turnover characteristic of the days previous to operation. Hence $1\frac{1}{2}$ nephrectomy modified the intake and output of water, but
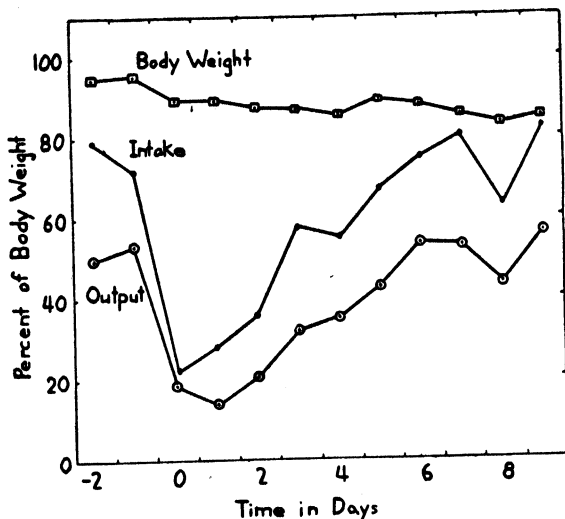

Fig. 1. MEAN BODY WT. and rates of fluid intake (2.6% milk solids) and of urinary output, in % of body wt/24hr., in 4 rats from which $1\frac{1}{2}$ kidneys were excised on zero day.

only temporarily. The body weight progressively diminished during the days of observation, but no more rapidly than in unoperated rats on this dilute diet. As previously shown (1), maximal water intake prevailed only when the rats were furnished a mixture of milk which was so dilute (2.6% of solids) that maintenance of body weight failed by a slight margin.

Further results can be pictured in terms of fluid intakes, since urinary outputs paralleled them on each experiment. The disparities between fluid drunk and urine collected were due chiefly to incomplete catchment of urine, some of it evaporating before it ran down the funnel of the metabolism cage. It is seen (fig. 2) that excision of one kidney (*Group A*) diminished the intake of fluid but slightly and for only a single day at operation. Excision of half a kidney (*Group C*) likewise diminished the intake slightly for a single day; subsequent excision of the whole remaining kidney diminished it for two days. Unoperated controls showed a suggestion of diminution for a single day, due to the fact that they were denied food and drink for the duration of the surgical operation and recovery period in the other individuals.

In sum, removal at one operation of $1\frac{1}{2}$ kidneys produced the only marked diminution of water turnover and that diminution lasted only six days.

The above experiments show that unilateral operations upon kidneys diminish water exchanges only on the day of operation. After bilateral operations on one day, six or seven days are required for recovery. Evidently the reduction of renal mass does not itself decrease the turnover of water (as in $\frac{1}{2}$ + 1 nephrectomized) but the immediate sequelae of operation do. It is well known (2, 3, 4) that excision of a kidney leads to inflammatory processes along with hyperplasia of proximal tubular epithelium in the other kidney. Much renal insufficiency temporarily prevailed, most of which effectively cleared up within seven days.
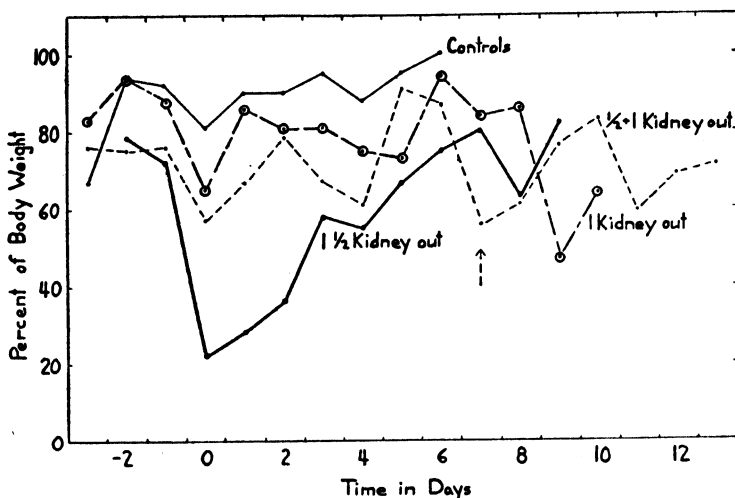

Fig. 2. MEAN RATES of fluid intake (2.6% milk solids) in % of body wt/24 hr., in rats subjected to 3 extents of nephrectomy upon zero day. Rats $\frac{1}{2}$ + 1 nephrectomized were also subjected to the second portion of nephrectomy upon the 7th day (at arrow). Each point is the mean of 12 in controls, 8 in $\frac{1}{2}$ + 1 nephrectomized, 4 in $1\frac{1}{2}$ and 4 in 1.

The severity of the operation was indicated by the fact that 43 rats underwent excision of $1\frac{1}{2}$ kidneys in order that 16 could survive until planned autopsy was in order. The other 27 died at two to eight days following the operation, with signs of uremia. In them, it is believed, recovery of the reorganizing renal tissue was too slow to allow the rat to survive. In some, blood supply to the remaining renal tissue may have been occluded. Of 14 rats $\frac{1}{2}$ + 1 nephrectomized, 5 died, 3 of them after a single operation, and 2 after the second operation. Hypertension is known to develop gradually during the first two months after this operation (5, 6). Therefore the lethal effect seems to be only partly related to the amount of tissue excised; instead, it is related to the immediate tissue damage done at any one operation.

Water Diuresis. Control rats were tested repeatedly to find whether water excretion was more rapid after they had experience in water diuresis. The standard water administration (6 doses) was employed. Figure 3 shows that after several tests at semi-weekly intervals the rats excreted the water faster than in the first test.

As a result, the body weight did not reach so high an excess. Maximal rates of excretion seemed to be reached in the third test of each individual (table 1) and were not enhanced further in as many as eight repeated tests. These tests illustrated the fact that rats adapt to water excess by excreting the water faster, as was reported by Liling and Gaunt (7). In the present tests it was apparent that the faster excretion of water directly prevented the accumulation of so great an excess of water in the body and, consequently, avoided a part of the tendency for toxic effects of water excesses to occur. Most noteworthy is the fact that the water diuresis after adaptation was much more prompt in its onset and did not require the slow development which was characteristic of the first tests. The diuresis may have been reflexly in-

TABLE 1. MAXIMAL WATER LOADS AND MAXIMAL URINARY FLOWS IN RATS GIVEN BY STOMACH AT 30-MINUTE INTERVALS 6 DOSES OF WATER EQUAL TO 5 PER CENT OF BODY WEIGHT

SERIAL TEST	DAYS AFTER NEPHRECTOMY	NUMBER OF TESTS	MEAN MAXIMAL LOAD	ITS STANDARD ERROR	MEAN MAXIMAL FLOW	ITS STANDARD ERROR
			% of wt.		% of wt/hr.	
Unoperated						
1		25	12.5	±0.7	6.0	±0.4
2		8	9.2	±1.0	7.0	±1.0
3		5	8.0	±1.1	8.8	±1.2
4		5	9.4	±1.5	8.6	±1.6
5		5	9.7	±1.7	7.2	±0.4
6		3	9.5	±1.0	8.0	±2.5
1½ Nephrectomized						
1	2–3	8	19.7	±1.9	3.0	±0.6
2	4–6	7	17.3	±1.5	4.4	±0.8
3	7–10	7	19.1	±1.8	5.0	±1.1
4	11–17	7	16.7	±1.8	3.8	±1.0
5	18–24	7	15.5	±1.5	3.8	±0.6
6	25–31	6	17.5	±0.5	5.0	±0.7
7	32–38	4	16.8	±0.3	5.4	±1.0

hibited somewhat in the first and second tests by the procedure of repeated stomach-tubing. It may be remarked (fig. 3) that the most rapid urine flows were in the periods after the last administration of water; this is the case even in the tests after acclimatization to water administration. This phenomenon also suggests that there may have been a release from inhibition where manipulations of the rat ceased.

Individuals 1½ nephrectomized (Series II) were in all stages of adaptation, having had from 0 to 8 previous tests. After operation they were tested usually on the second, fifth and ninth days and subsequently at weekly intervals (table 1). Some individuals were too ill to be used for test; these died. In addition, those with the lowest rates of excretion during water tests soon died before a second test could be made. There was no evidence that the water administrations as such prevented survival.

In first water tests after 1½ nephrectomy (fig. 4), excretion of water was much

slower than before operation (about $\frac{1}{4}$ as rapid) and water accumulated to an average of 20 per cent of the body weight. Subsequently the rate and promptness of diuresis increased, so that in the fifth tests (at 18–24 days postoperatively) the rates were about one-half and, later, two-thirds of those characteristic of the same individuals before partial nephrectomy. The higher rates of excretion then prevented so great an accumulation of water in the body.

Apparently one fourth of the renal tissue could on the average excrete urine one-half or two-thirds as fast as all the renal tissue. Individual tests showed as much as four fifths of the mean excretion manifested by intact kidneys. Four rats with $1\frac{1}{2}$ kidneys excised were kept for $1\frac{1}{2}$ to 3 months and successive tests tended to show progressive recovery of maximal urinary flow. The results suggested that the limit of functional recovery was then approximately reached. It may be concluded that while complete absence of renal tissue would, of course, prevent water excretion, less

Fig. 3. URINARY OUTPUT in relation to water load in intact rats. First test, mean of 25 individuals; 5th test, mean of 5 individuals. In each test water equal to 5% of the body wt. was placed in the stomach each half hour for 6 doses. Points represent the mean water retention and mean rate of excretion during each half hour period; open points during increasing water loads, solid points during subsequent decreasing loads.
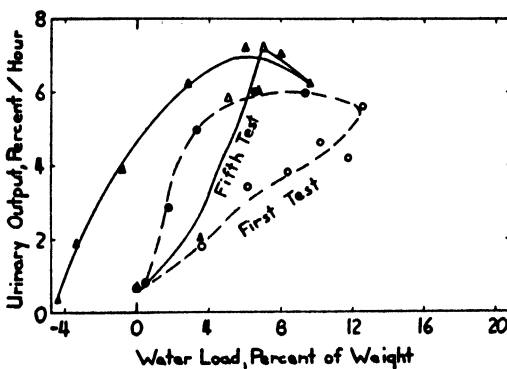
than one half of the tissue is sufficient for a maximal water diuresis at rates approaching the rates of excretion possible in intact kidneys.

The increase in rate of water excretion that followed recovery from partial nephrectomy was not appreciably due to the above adaptation process in the water test. The increase was about as great in previously adapted rats as in fresh ones. Presumably it was chiefly concerned with reorganization of functions in the remaining renal tissue. It was apparent up to 24 days after operation, which was a much longer period of increase than was visible in the drinking tests (fig. 1). Evidently the turnover of fluid ingested in the form of 2.6 per cent milk solids was limited by the maximal rate of water excretion only up to the seventh postoperative day; thereafter about half the maximal rate characteristic of the intact rat prevailed and was sufficient to maintain the turnover. Nevertheless, to maintain that turnover the maximal rate (3%/hr.) had to be approximately maintained by the rat in every hour of the day and night.

It might be supposed that the large excesses of body water forced upon the rats in six doses of 5 per cent of the body weight were too large for optimal excretory functioning. Other tests therefore were done in which three doses of 5 per cent each were given (fig. 5). In control rats the maximal rates of excretion attained were 5 per cent/hr., instead of the 6 to 8 per cent/hr. after six doses. Two days after $1\frac{1}{2}$

nephrectomy, maximal diuresis was about 3 per cent/hr., which was very close to the mean rate found after six doses (fig. 4). The total effect of nephrectomy upon water diuresis was therefore manifested to about the same degree whether three or six doses of water were administered and whether maximal water loads were 20 per cent or only 10 per cent of the body weight.

Current views as to how water is excreted in mammalian kidneys emphasize the enormous rates of believed capsular filtration. According to these views, reduced renal tissue would have no difficulty in forming urine rapidly. The estimated rates of filtration (maximal creatinine clearances) in normal rats are reported as 90 per cent of the body wt/hr. (8). Unless the filtration rate increases in the residual fraction of renal tissue, as is altogether likely, the remaining one fourth of kidney substance was during the water test excreting urine equal to one fifth of its original filtra-
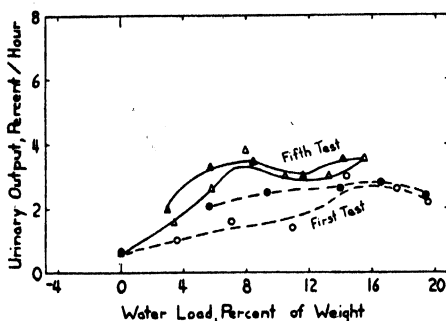

Fig. 4 (*left*). URINARY OUTPUT in relation to water load in nephrectomized rats. Procedure same as in fig. 3. First tests (8) were done 2 or 3 days after 1½ nephrectomy; 5th tests (7) were 18 to 24 days after operation.
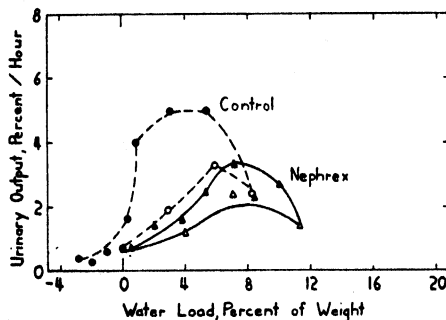
Fig. 5 (*right*). URINARY OUTPUT in relation to water load in rats given 3 doses of 5% of wt. of water; otherwise procedure was same as in fig. 3. Control tests (11) were before operation; other tests (4) were 2 to 3 days after 1½ nephrectomy.

tion flow (22%/ hr.). In anesthetized rabbits under sulfate diuresis it is estimated that urine flow may equal two thirds of the capsular filtration rates and, under bichromate nephritis, may even equal them (9). Solutes that are ordinarily 'reabsorbed' appeared in the urine under those conditions. In the rats studied here, neither glucose nor protein appeared in the diuretic urine more frequently than in ordinary urine.

The experiments show clearly that the maximal rate of water excretion in the normal rat is not wholly limited by the amount of renal tissue. The flow or pressure of the blood may be limiting or the rate of water absorption from the intestine may be limiting. The latter would presumably be unaffected by partial nephrectomy, and if limiting, would not lead to the convulsions which occur; the former may adjust within a brief period of time after operation.

The ordinary rates of water turnover by rats as a result of 1½ nephrectomy remain to be mentioned. Five individuals were intensively studied for 3 days before operation and 17 days after it. Food intakes were less for 3 days after operation, but water intakes were less only upon the day of operation and urinary output paral-

leled them. Hence, though others have observed polyuria (5, 10), we found no important or significant change in metabolism of solids or of water beyond the three days after nephrectomy. Whether dietary or other factors are responsible for the difference is not clear.

Water Intoxication. Convulsions were induced when large quantities of water were retained in rats, as in other mammals. In the present series, convulsions were absent in all but one of 54 tests before nephrectomy (25 individuals). They occurred in 14 of 54 tests after nephrectomy (14 individuals). Any one individual might convulse in some tests and not in others. Somewhat more water was retained in the nephrectomized individuals (16–20% of the body wt. at 3 hr. of the test) as compared with the intact rats (10–13%). At 16 to 22 per cent retention lies the usual threshold for convulsions in rats. It should also be recognized that other factors than water content or dilution of body fluids enter into the induction of convulsions, since marked species differences prevail.

Hemoglobin sometimes appeared in the urine during water tests, with equal frequency in the controls and in the nephrectomized. Hence hemoglobinuria did not result from the state of the kidneys nor from the extent of the water excesses present in the body as a whole; probably it was produced by the dilution of blood during the process of water absorption from the intestine. Aside from the days of water tests, blood and protein appeared in the urine almost exclusively on the days of surgical operations and were evidences of local damage.

Renal Hypertrophy. It has frequently been supposed that prolonged exaggerations of water turnover lead to renal hypertrophy. As evidence for this, the large kidneys sometimes found at autopsies of habitual beer-drinkers are cited. Rats that received only 2.6 per cent of milk solids in water were often excreting their body weights of urine every day. Some 25 of them (not nephrectomized) were killed, their kidneys were weighed and compared on the basis of body weight with the standard series reported by others (11). No appreciable difference was found (table 2). It is concluded that rapid turnover of water is not a stimulus to hypertrophy of the kidneys.

Evidence of renal hypertrophy inevitably involves a knowledge of changes of body weight and of renal growth that occur during any dietary regime. Upon the above dilute-food regime the rats were slowly losing weight. It might be that the kidneys did not grow smaller as the body shrank and that the body weight characteristic of the day upon which the regime started should be used for prediction of kidney weights. After certain types of weight diminution due to dietary restriction, however, it has been ascertained that the kidneys diminish proportionally in weight (12). Actually the body weight diminutions were sufficiently small (table 2) so that the hypertrophy would not be significantly different if the initial body weights had been used in predicting renal weights.

Partial nephrectomy is well known to induce hypertrophy of the remaining renal tissue. Would this hypertrophy be greater in rats having high turnovers of water? The extent of hypertrophy was accurately judged in the 1-nephrectomized rats by comparing the weight of the kidney obtained at autopsy with the weight of the kidney first excised at operation. The hypertrophy was moderate (table 2) and of the same

magnitude as in adult 1-nephrectomized rats maintained upon diets of low water content but of about the same protein contents (13, 14).

After 1½ kidneys had been removed, the hypertrophy could be estimated only by adding the weight of the half kidney removed at operation to the weight of tissue found at autopsy. The average percentage hypertrophies were 15 per cent in the rats operated in two stages and 30 per cent in the rats operated bilaterally at one

TABLE 2. MEAN WEIGHTS AND WATER CONTENTS (WITH STANDARD ERRORS) OF THE KIDNEYS OF RATS UPON DIVERSE REGIMES

REGIME	NO. OF ANIMALS	MAX. BODY WT.	BODY WT. AT NEPHRECTOMY	WT. OF ONE KIDNEY	WATER IN TISSUE	HALF KIDNEY WT.	WATER IN TISSUE	REMAINING KIDNEY WT. AT AUTOPSY	WATER IN TISSUE	RENAL HYPERTROPHY
			gm.	gm.	%	gm.	%	gm.	%	%
Dry milk diet, controls	7	186.5	180.3	0.750	76.0 ±1.22					
Dry milk diet, 1½ kidneys out	7	234.9	228.6	0.893	75.2 ±1.47	0.386	76.3 ±0.84			
	11[1]	206.4	198.4	0.844	75.8 ±1.67	0.400	78.7 ±0.56			
2.6% milk, controls	25	173.5	153.6	0.729	76.7 ±0.57					
2.6% milk, 1 kidney out	9	178.0	164.5	0.723	76.7 ±1.88			0.788 (9)	75.2 ±1.20	13.1
2.6% milk, (½ + 1) kidneys out	8	185.3	166.4	0.707	75.6 ±0.82	0.308	77.5 ±0.59	0.499 (8)	78.1 ±0.81	14.6
	2[1]	158.5	127	0.578	78.8	0.225	77.3			
2.6% milk, 1½ kidneys out	9	202.7	189.1	0.820	77.0 ±1.31	0.344	76.7 ±2.60	0.725 (8)	76.7 ±0.17	29.6
	16[1]	197.3	178.3	0.766	76.9 ±0.49	0.339	77.2 ±1.68			

[1] Rats died prematurely.

etherization. Previous investigators (5) carried out 75 to 85 per cent excision of kidneys in rats but did not attempt to estimate the resulting hypertrophies.

It is plain that after partial nephrectomy, the remaining renal tissue undergoes no more hypertrophy with the stimulus of rapid turnover of water than without it. A hypertrophy of 30 per cent by weight is insufficient, in fact, to account for the amount of function manifested during water diuresis and it can be concluded that water diuresis was not greatly limited by the amount of renal tissue. It is probable that more extensive hypertrophy occurs in certain proximal tubular structures of the kidneys, as pictured by Oliver (4) and Rollason (15), but not in the number of nephra (16, 17).

The water contents of excised renal tissues were ascertained (table 2). On logarithmic grids the lines drawn through the points of absolute water contents are parallel to those drawn through the points of absolute kidney weights. Hence the relative water contents were not different before and after hypertrophy, as the tabulated means show. In hypertrophied kidneys some investigators have reported that the water fraction is increased (14); others that it is unchanged (3, 21).

Slight increases in relative water content were reported in kidneys taken from rats killed while they had water diuresis (18). It is possible that direct comparisons between our animals in high water turnover and those consuming dry food would have demonstrated a barely significant increment of water in the renal tissue.

<div align="center">COMMENT</div>

The rate of forced water ingestion was reduced for a few days following excision of more than one kidney. Evidently the excretion of water was then retarded, leading to an immediate inhibition of intake when only dilute food was available. As the reduced renal tissue recovered from the operation, the intake was restored to its initial rate. This inhibition constitutes evidence that a slight accumulation of excessive water in the body is sufficient to discourage intake of water, even though the urge to obtain food is somewhat increased at the same time. Some day it will be known just how an increase of body water content may suppress the urge to drink.

The rate of water excretion, it is shown, depends upon the amount of functioning renal tissue to only a limited extent; it is further limited temporarily when the functioning is upset as a sequel of surgical manipulation. Once the renal tissue has recovered from the immediate effects of operation, one fourth of the renal bulk is quite sufficient to carry on either maximal forced turnover of water or half-maximal forced water diuresis. While a marked reduction of renal bulk could limit water diuresis, another correlative of functional capacity than mere bulk of tissue may some day be identified.

Maximal rates of urine production have now been obtained in normal rats after adaptation to repeated administrations of water. These rates average 8 per cent of the body wt/hr. The shape of the curve, relating water excess to rate of excretion (fig. 3), is sensibly linear up to 4 per cent of the wt/hr.; at higher rates it tends to be independent of water load. Hence there is reason to believe that still higher loads (above 15% of the wt.) will not induce faster diuresis. Loads of 20 per cent usually induce convulsions in rats; these convulsions are not lethal, we find. The occurrence of convulsions is not correlated with the period of time since nephrectomy and hence not with the amount of arterial hypertension.

An intermediary regulator of renal hypertrophy is believed to be the anterior hypophysis. When it has been removed, unilateral nephrectomy no longer leads to renal hypertrophy (19); instead, the kidneys diminish in size and possibly in function (20). The chief factors that exaggerate the hypertrophy after partial nephrectomy in rats are: youth, high protein intake and testosterone administration (21). All are believed to represent a high intensity of protein metabolism, which might in turn excite the intermediaries. These same factors accelerate the enlargement of the kidneys without nephrectomy. To many other factors that have been found not to

· induce renal enlargement, continuous rapid water excretion may now be added. The present results confirm in this respect the negative results obtained in the attempts of Hinman and Belt (22) and Chanutin and Ludewig (23) to influence renal hypertrophy or water retention by injecting considerable volumes of saline or urea solution each day.

SUMMARY

After three to five repetitions of excessive water administration in normal rats, water diuresis was characterized by less delay in onset and greater rates of output. Maximal water diuresis was greatly diminished when $1\frac{1}{2}$ kidneys were excised. Within four weeks after operation water diuresis recovered to be, on the average, two thirds as rapid as before it. Water ingestion, that was forced by the method of furnishing only dilute food, was diminished only for one week following $1\frac{1}{2}$ nephrectomy. Half or one nephrectomy did not have any appreciable effect upon water ingestion. Following $1\frac{1}{2}$ nephrectomy, the hypertrophy of the remaining renal tissue was no greater upon the regime of forced water than without it. Evidently prolonged excessive excretion of water was not a factor in determining the amount of hypertrophy. The water content of the kidneys was not significantly increased by the forced water regime nor by the hypertrophy superimposed upon it.

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MYOGRAPHIC STUDY OF THE CAT'S HEART: EFFECT OF CHANGES IN VENOUS RETURN AND IN PERIPHERAL RESISTANCE ON VENTRICULAR CONTRACTION

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MYOGRAPHIC studies of ventricular contraction have been used to demonstrate that under certain conditions the heart contracts more or less forcefully (1, 2). For the most part it has been overlooked that the heart resembles most closely an elastic hollow sphere as first pointed out by Bayliss and later by Gesell (3, 4). The fact that the muscle fibers of the heart travel in different directions not only on the surface but in the depth of the myocardium further complicates matters. Moreover, with dilation the heart wall becomes thinner and the muscle fibers change their relationship to each other; most fibers are lengthened some conceivably are shortened. Finally the tension in the different layers of the heart wall is not equal in all layers. Particularly in the left ventricle it has been shown that the tension is greatest near the endocardium gradually diminishing towards the epicardium (5). With changes in heart size this gradient of tension is altered as the wall becomes thicker or thinner and this might be reflected in myographic measurements.

METHODS

In all the experiments cats (8 female, 7 male) weighing between 2 and 5 kg. were used. Anesthesia was intraperitoneal dial. The chests were opened and artificial respiration was given by means of an automatic pump. The venous pressure measurements were made by direct cannulation using a saline manometer. Heparin in the saline kept the cannulae free of clots. Arterial pressure was recorded from the carotid artery by use of a Hürthle Manometer in most of the cats and by a Hamilton Manometer with a cannula in the aorta in the others. The contractile force of both the right and left ventricular muscle was recorded by three different myographic techniques in separate experiments. Only the second method will be discussed in detail since the others have previously been described.

METHOD I. *Cushny's Weighted Myograph.* Essentially the same apparatus was used and techniques followed as described by Walton and Brodie (1). This consisted of an ordinary Cushny's myograph to which was added a calibrated steel spring. In practice by increasing the tension on the spring the movable lever was weighted to the exact point where motion of muscle no longer occurred. This point was called the isometric systolic tension (I.S.T.). In our experiments the myographs were sewn to both the right and left ventricles. The one for the right ventricle was placed on the anterior surface of the heart; the direction of contraction was towards the right shoulder region. The left Cushny myograph was placed on the left lateral wall of the heart and in this in-

stance the direction of contraction was parallel to the longitudinal axis of the cat. In all instances the m ovable lever of the myocardiograph was attached as far as practicable from the apex of the heart.

METHOD II. *Dynamograph.* The objection frequently raised with the Cushny type of myograph is that with changes in heart size the lever arms measure different areas of muscle contraction. To overcome this difficulty a new type of myograph was designed (fig. 1). A lucite capsule whose lower surface enclosed an area of exactly one square centimeter was applied to the anterior surface of the right ventricle by means of suitable clamps. In the center of the circular aperture of the capsule a silk ligature was sewn to the heart wall. The upper end of the thread was tied to an isometric spring which was made of 31 turns of B&S 20-gauge piano wire in a ⅜″ diameter closely wound spiral. Another length of thread extended from the lower end of the spring over a pulley either to a lever system for kymograph recording or a mirror for optical recording. It can be seen how the myograph works by referring to figure 1. During diastole when there is no tension on the wall of the heart the muscle bulges into the capsule. In systole the heart wall becomes tense, assumes a spherical shape and resists distortion of its surface hence little muscle can be pulled into the capsule. It is this in-and-out motion which is recorded. The spring and recording system is calibrated for each experiment by hanging weights on the lower end. Once set up the desired initial tension can be secured by raising or lowering the spring. In practice it was found that 10 gm. was the least initial tension compatible with smooth recording. The heart muscle remained well nourished and no local cyanosis was noted in five consecutive experiments. The typical records obtained with this device will be discussed in connection with the results. It should be obvious that the main advantage of this myograph is that the force of contraction of one square centimeter of heart muscle is measured despite changes in contour or size of the heart.

METHOD III. *Intramyocardia Pressure.* This method has been extensively described before and consists of recording optically the changes in tension of an artery segment imbedded in the wall of the left ventricle (5). Although this method has been criticized by Gregg the findings in this study are limited to comparative values in the same animal and for this purpose are reliable (6).
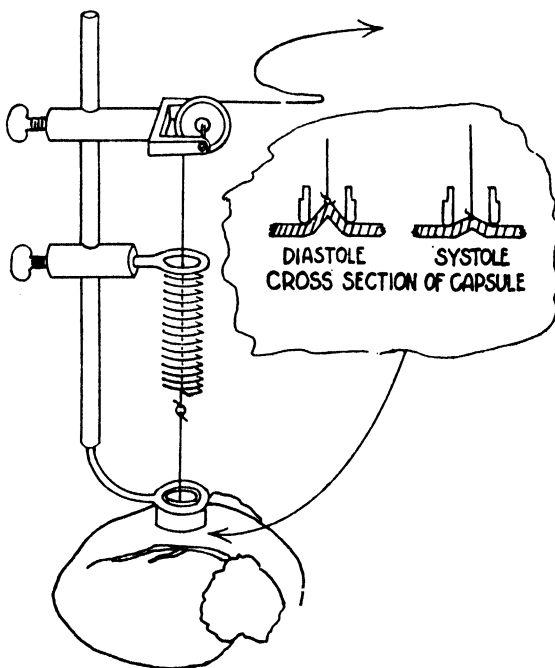
RESULTS

Unweighted Cushny Myograph. When 30 cc. of N saline is infused at the rate of 10 cc/min. the degree of excursion of the segments of muscle under study in both the right and left ventricle decreases (fig. 2). This occurs despite the rise in arterial pressure. It does not occur if the infusion is small or the animal is dehydrated, i.e. enough fluid must be given to increase the size of the heart appreciably. A decreased venous return caused by clamping the inferior vena cava results in increased excursion of the heart muscle despite a fall in arterial pressure (fig. 3). Again this result will not be obtained if the decreased venous return does not cause a decrease in cardiac size, i.e. if the heart is at a minimum size initially. These results duplicated in four separate experiments show that an increase in cardiac diameter is associated with decreased excursion of the muscle fiber and a decreased diameter with increased excursion.

Isometric Systolic Tension. Appropriate weighting of the Cushny myograph as in the technique of Walton and Brodie (1) permitted a determination of the isometric systolic tension (I.S.T.). These results obtained in one of three typical experiments are summarized in tables 1 and 2. With increased venous return the I.S.T. falls from a control of 18 gm. in the right ventricle to a low of 11 gm. in 2 minutes, recovery taking place in 15 minutes. During this period there is a rise in venous pressure and in arterial pressure (table 1). Changes of the same order occur in the left ventricle. Similar measurements during decreased venous return (clamping

the inferior vena cava) show the opposite result. In this case the clamping was done at intervals so as to avoid anoxia of the heart muscle. Table 2 shows that there is a slight but definite rise in I.S.T. when the return of blood to the heart is decreased, more marked in the right ventricle as compared to the left. The reason for this is felt to be because of the difficulty of properly applying the myograph to the posteriorly placed left ventricle rather than to actual differences in response of the two ventricles. This occurs despite a marked fall in arterial pressure and venous pressure. Again it is inferred that the major cause for these observed changes in I.S.T. is the change in cardiac diameter.

Fig. 1. DYNAMOGRAPH. A special type of myograph which permits measurement of contractility of 1 sq. cm. of intact heart muscle, in spite of changes in cardiac diameter.

Dynamograph Records. This instrument gave relative measurement of the force of contraction of one square centimeter of muscle on the surface of the right ventricle, in spite of changes in cardiac size. Figure 4 shows the results obtained in one of five typical experiments. Starting on the left is the control period where the tension is 30/gm/sq. cm. diastolic (load tension) and rises to 68 gm/sq. cm. in systole. The arterial pressure is 65 mm. Hg. With an infusion of 30 cc. N saline, 10 cc/min. the diastolic tension has risen to 37 gm/sq. cm. and the systolic tension has fallen to 60 gm/sq. cm. at the end of 30 seconds. Hence the force developed during contraction has changed from 38 gm/sq. cm. in the control period to 23 gm/sq. cm. during venous infusion. Again this occurs in spite of arterial pressure rise. Similar changes occur in tension but in the opposite direction when the venous return is decreased by clamping the inferior vena cava (fig. 4). Thus this method gave simi-

·lar qualitative results as the weighted Cushny myograph but under conditions where the surface area of the muscle under test was kept constant.

Intramyocardial Pressure. It was felt desirable to check the paradoxical results obtained with the Cushny myograph and the dynamograph with a different method of recording. Figure 5, *A* and *B*, shows the result obtained in a typical experiment with the infusion of 35 cc. of N saline at the rate of 10 cc/min. Control arterial pressure was 87/68 mm. Hg (fig. 5 *B*). At the termination of the infusion the pressures were 97/62 and 78 mm. Hg respectively (not shown in fig. 5). In this experiment the carotid artery segment was located just to the left of the ramus desendens anterior artery and at one-third the depth of the left ventricular wall measuring from the epicardial surface. Thus this experiment permits the conclusion that the lateral tension developed during systole in the wall of the left ventricle decreases with increases in cardiac diameter brought about by augmented venous return.

Initial Tension and Force of Contraction of Ventricular Muscle. The results thus far conclusively demonstrate that increases in venous return with the consequent increase in cardiac diameter (initial length) are attended by a decreased ability of the myocardial muscle to shorten (as measured by these techniques). The question may well be asked what does serve as a stimulus for augmenting myocardial contractility? The dynamograph is a suitable instrument to test the effect of increased initial tension on the force of contraction. Figure 6 shows one such experiment. Arterial pressure remained relatively constant at a mean of 90 mm. Hg so it may be assumed that the state of the myocardium remained constant throughout the experiment. When the dynamograph diastolic tension was set at 10 gm/sq. cm. the systolic tension developed was 40 gm/sq. cm. Increases in diastolic tension to 27 and to 40 gm/sq. cm. resulted in further rises in systolic tension of 75 and 100 respectively. There is no doubt that an increased initial tension causes enhanced cardiac muscle contraction.

Intraventricular Tension and Force of Contraction. Clamping the aorta or pulmonary artery provides a convenient means of raising the intraventricular tension or initial tension. In figure 5, *C* and *D*, the effects of clamping the aorta on intramyocardial pressure are shown. In figure 5 *C*, the control blood pressure was 93/70 mm. Hg and the intramyocardial pressure 80 mm. Hg. Clamping the aorta raised the intramyocardial pressure to 165 mm. Hg. Similar qualitative results were obtained using the dynamograph and the weighted Cushny myograph on the right ventricle but these are not included for the sake of brevity. It must be pointed out that raising the initial tension in this manner also increases the initial length as the heart·regularly dilates with this procedure. However it has been demonstrated above that increases in initial length do not augment the force of cardiac contraction (measured with the myographs) and these last experiments indicate the importance of initial tension.

<center>DISCUSSION</center>

The perfect myograph would permit mensuration of the force of contraction of a constant volume of heart muscle despite changes in heart radius. Such an instrument has not been devised. The dynamograph used in this study while capable of
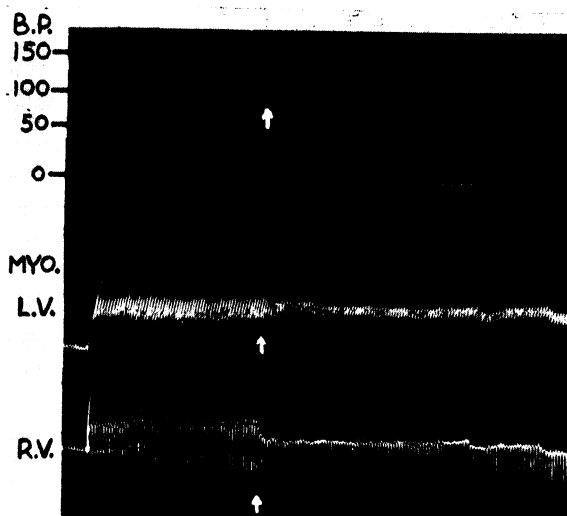
Fig. 2(*upper*). INCREASED VENOUS RETURN, unweighted Cushny myograph. From above downwards, arterial pressure, myograph left ventricle and right ventricle. Note the decrease in excursion with the onset of increased venous return (*arrows*).
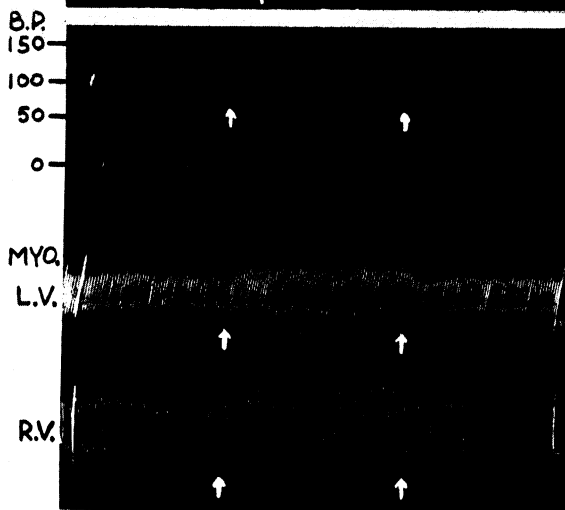

Fig. 3 (*lower*). DECREASED VENOUS RETURN, unweighted Cushny myograph. Tracings as in fig. 2. Note the increase in excursion with the onset of decreased venous return brought about by clamping the inferior vena cava (*arrows*).

Fig. 4. DYNAMOGRAPH RECORDS. *Top*, blood pressure. *Bottom*, tension of 1 sq. cm. of right ventricle. *Left*, note that with increased venous return the tension decreases from 38 (68 minus 30) to 23 (60 minus 37) gm./sq. cm. *Right*, the opposite changes occur with decreased venous return.
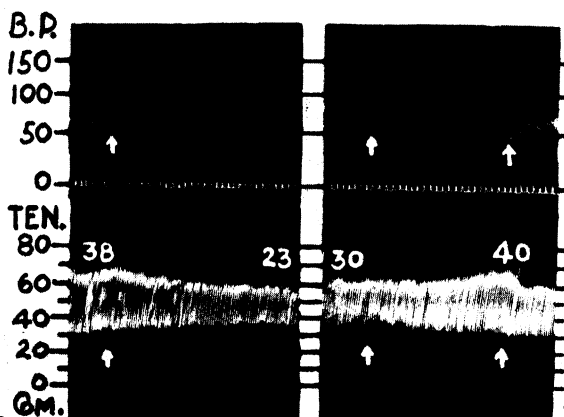
measuring a constant area of muscle still does not account for changes in thickness of the heart wall. Cushny's myograph suffers from the added disadvantage that the distance between the recording arms changes as the diameter of the heart is

Fig. 5. INTRAMYOCARDIAL PRESSURE measured from an imbedded carotid artery segment in the wall of the left ventricle (see text) related to aortic blood pressure. *A*, control period; *B*, during increased venous return. Note that the lateral tension in the left ventricle decreases from 90 (290 minus 200) to 60 (260 minus 200) mm. Hg in spite of a constant aortic arterial pressure. Note also that while heart rate is constant the systolic period increased. Cardiac diastolic diameter had increased. *C*, another control period; *D*, during clamping of the aorta distal to the innominate artery. Note in this case the great increase in lateral tension.
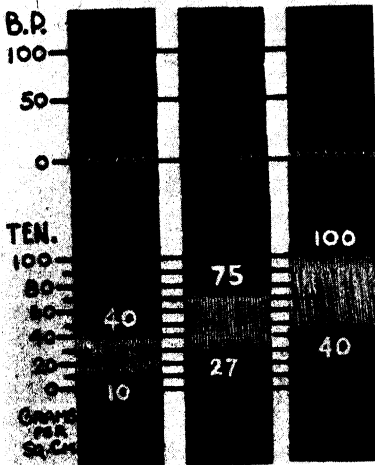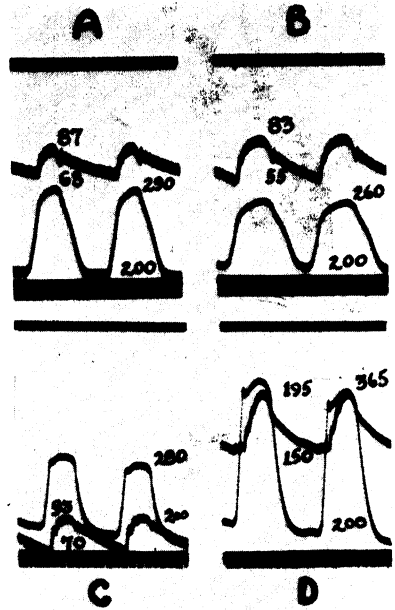
Fig. 6. DEMONSTRATION that an increase in initial tension causes increased contractility of the intact right ventricle. *Top*, arterial pressure; *bottom*, dynamograph. Note that with increases in initial tension from 10 to 27 to 40 gm./sq. cm. the maximum systolic tension developed is 40, 75 and 100 gm./sq. cm. respectively. Arterial pressure has remained constant throughout.

altered. An imbedded artery segment also obviously does not have a constant volume of muscle acting upon it under conditions where the heart wall varies in thickness. These considerations preclude using the myograph in any quantitative study of heart muscle contractility.

<div align="center">TABLE 1</div>

TIME	ISOMETRIC SYSTOLIC TENSION, GM.		CM.H₂O VENOUS PP.	MM. Hg ARTERIAL PP.
	R.V.	L.V.		
Control o	18	30	2.1	50

<div align="center">*Infusion 25 cc. saline, 10 cc. min.*</div>

sec. 10	12	23	2.3	60
20	11	22	2.5	65
40			3.0	70
60	11.5	22	3.2	70
80	12	23	3.4	73
180	11	22	3.6	80
min. 9	14	24	3.0	78
15	16	29	2.5	75

R.V. = Right ventricle.   L.V. = Left ventricle.

<div align="center">TABLE 2</div>

TIME, MIN.	ISOMETRIC SYSTOLIC TENSION, GM.		CM. H₂O VENOUS PP.	MM. Hg ARTERIAL PP.
	R.V.	L.V.		
Controls				
o	15	36	2.7	73
2	17	40	2.5	73
4	16	32	2.2	74
6	17	31	2.3	75
Average..............	16.2	34.7	2.4	74
During decreased venous return				
8	18	33	1.5	10
10	21	34	1.3	12
12	23	37	1.3	15
14	24	39	1.8	25
Average..............	21.5	35.7	1.5	15.5
Controls				
9	18	30	2.8	80
11	16	27	2.4	85
13	16	28	3.0	75
Average..............	16.6	28.3	2.7	80

R.V. = Right ventricle.   L.V. = Left ventricle.

Qualitative deductions may be permitted from myographic study provided certain physical relationships of the heart are kept in mind.   The heart is a hollow globular

organ most closely resembling a hollow elastic sphere such as a soap bubble (3, 4). When the resting or diastolic diameter of such an organ increases, the volume contents increase as the radius cubed divided by three while the surface area only as the radius squared. To now expel its contents the muscle need not shorten through as great a distance (4). Our results show that this is true for the cat's heart under conditions of increasing venous return (figs. 2 and 3). The total volume of muscle composing the wall of a ventricle cannot change acutely. With an increase in cardiac radius the wall must perforce become thinner since it is spread over a greater surface area. This has the effect of showing a decreased contractility when studied myographically. The reason for this is obviously the fact that with the increase in cardiac radius a relatively smaller amount of muscle is being measured by the myograph. Our results conform perfectly to this interpretation (tables 1 and 2, figs. 4 and 5).

On the other hand, when the initial tension is altered either locally or by increasing peripheral resistance our results show uniformly that the ability of the heart muscle to shorten is enhanced (figs. 5 and 6). This result obtains even when the radius of the heart is increased because of the increased load of a greater peripheral resistance. This would seem to indicate that changes in initial tension are capable of inducing a positive inotropic effect on the muscle fiber. In contrast changes in initial length have per se no effect on the state of contractility itself. Heart performance is altered merely because of the physical peculiarity of a hollow sphere, i.e. volume/surface area ratio is changed so that the heart wall has a better or worse mechanical advantage.

Finally it should be pointed out that any type of myographic study of heart muscle should take cognizance of the results of such experiments as these. For example the comparison of different drugs on myocardial contractility by a myographic technique should be attended by observations of cardiac diameter and of changes in peripheral resistance. It is obvious that if a drug has the effect of increasing cardiac radius it will also have the apparent result of decreasing contractility. Also a drug which increases peripheral resistance will raise the initial tension and the myograph will record an increase in contractility which may not be a direct myocardial effect.

SUMMARY

Using three different myographic techniques the nature of cardiac contraction was studied during increased and decreased venous return and clamping of the aorta. Cats with the chest opened but with intact circulations were used. Analysis of the results was made on the basis that the heart resembles most closely an elastic hollow sphere. The results justify the following conclusions: 1) During increased venous return a segment of heart muscle under study contracts through less distance. The opposite is true for decreased venous return. 2) During increased venous return a segment of heart muscle contracts less forcibly. The opposite is true for decreased venous return. 3) During clamping of the aorta and also with increases in initial tension induced locally with a special myograph (dynamograph) a segment of heart

muscle contracts more forcibly. Therefore, increases in initial tension cause the heart muscle fibers to contract more forcibly.

These results would seem to indicate that the ability of the heart muscle fiber to shorten is not altered by changes in initial length but is altered by changes in initial tension. In the former instance work output is changed merely by a shift in the volume/surface area ratio of heart contents to heart wall. In the latter instance work performance is increased or decreased by an actual change in ability of the muscle fiber to shorten.

The artifacts and mechanical paradoxes of myographic recording were pointed out and discussed. Qualitative deductions are permitted only when concomitant changes in heart radius and peripheral resistance are taken into account.

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RIGHT AND LEFT HEART FAILURE: UNILATERAL RISES IN RIGHT AND LEFT AURICULAR PRESSURE IN HYPERVOLEMIC CATS FOLLOWING NEAR LETHAL DOSES OF QUINIDINE, AURICULAR FIBRILLATION AND EPINEPHRINE

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O PINION is divided as to the exact mechanism responsible for the elevated venous pressure of congestive heart failure. It is generally agreed, however, that two factors most commonly present in late congestive failure are a weakened heart and an increased blood volume. In animals and man there is strong evidence that simple acute increases in blood volume are not capable of producing prolonged elevations of venous pressure consistent with those found in heart failure (1–5). Likewise, experimental weakening of the myocardium has been found incapable of elevating venous pressure to significant levels (6, 7). Congestive failure with elevated venous pressure has been reported when both myocardial weakness and plethora have been induced in the same animal (8, 9). Yeomans *et al.*, however, have observed pulmonary edema in normal dogs during the height of massive rapid infusions (10).

Remarkably few experiments have been made in which both right and left auricular pressures have been measured simultaneously. Our aim in this investigation was to compare the changes in the right and left auricular pressure in acute heart failure produced by combining hypervolemia with myocardial weakness. The results in this report furnish further evidence that dissociation of the dynamics of the right and left sides of the heart can occur under appropriate conditions of hypervolemia, myocardial weakness and recovery.

METHOD AND PROCEDURES

Data were collected from 13 cats, 8 female and 5 male, weighing 2 to 3.5 kg. Anesthesia was induced with Dialurethane solution intraperitoneally in doses of 0.6 to 0.7 cc/kg. body weight. Right and left auricular pressures were recorded with the chest opened and with artificial respiration through a tracheal cannula. Freshly paraffined glass cannulae were tied into the right and left auricles and pressures recorded with saline manometers to which heparin was added. The pressures were transcribed from the manometers to a smoked drum by Brodie Bellows which were calibrated before each experiment. Arterial pressure was recorded from the right carotid artery with a mercury manometer. Fluids and drugs were infused into the femoral vein. Electrodes attached to the right auricle were connected to a Thyratron stimulator having a frequency of 600 impulses/min. which generated current adequate to induce auricular fibrillation.
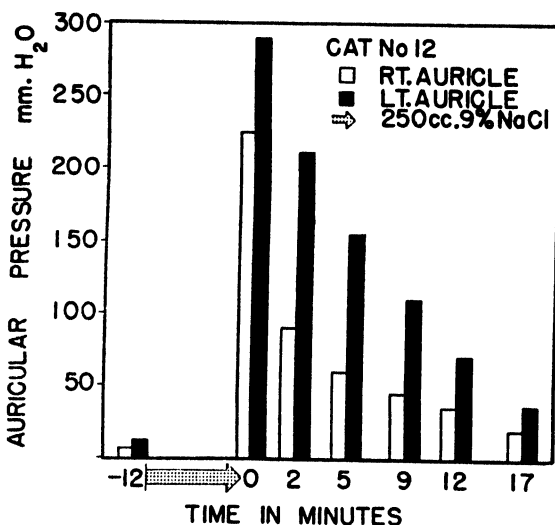
<center>RESULTS</center>

Normal Right and Left Auricular Pressures. In 13 open-chest cats right auricular pressure ranged between −10 mm. water and −43 mm. with a mean value of 17 mm. In the same animals left auricular pressure ranged between 12 and 70 mm. with a mean value of 42. Values for left auricular pressure are comparable to those found by others in dogs with open chests (13), and to measurements of pulmonary venous pressure in unanesthetized closed-chest dogs (14). A similar range of right auricular pressure has been reported in normal mammals with the chest intact (*dogs 5, 9, 11*), (*humans 11, 12*). In only one animal was left auricular pressure lower than right. The mean ratio of right to left auricular pressure was 1 to 2.5 which approximates results found by Dexter using a saline manometer in intact

Fig. 1. PRESSURE IN RIGHT AND LEFT AURICLE before and after acute hypervolemia. Control auricular pressures represented at −12 min. Zero time indicates level of auricular pressures immediately after estimated blood volume increased one-fold with physiological saline. Decline of auricular pressure to almost control levels plotted in succeeding 17 min.

dogs (15). The values obtained for resting auricular pressure in our cats, then, are in essential agreement with results of other workers.

Right and Left Auricular Pressure of Acute Hypervolemia. Landis and co-workers (5) and Yeomans *et al.* (10) have recently re-emphasized that simple massive increases in blood volume produced marked but only transient elevations of central and peripheral venous pressure with venous pressure dropping promptly after the infusion was discontinued. In one experiment in which left auricular pressure was measured it was found to rise more rapidly and to a much higher level than peripheral venous pressure (10).

Figure 1 illustrates a representative response to one-fold increases in estimated blood volume by rapid infusion of isotonic saline in 6 cats; 250 cc. of isotonic saline was given in 12 minutes. Right auricular pressure rose from 7 to 225 mm. water and left, from 12 to 290 mm.; 5½ minutes after the infusion was terminated right auricular pressure had dropped to 60 mm. and left, to 155 mm. Seventeen minutes after the end of the infusion auricular pressures were almost at control levels. Arte-

rial pressure dropped moderately at the onset of the infusion (115–100 mm. Hg) and had returned to control levels 17 minutes after end of infusion. This early drop has been noted by others (10) while Warren *et al.* (12) report that in normal human beings arterial pressure shows slight and random variations in response to infusion. Thus, as has been previously reported, acutely induced plethora produces marked but only transient elevations of right and left auricular pressure.

Bilateral Elevations of Auricular Pressure with Quinidine. It has already been demonstrated that large intravenous doses of quinidine produce severe myocardial impairment (16–19). This is characterized by a sudden drop in blood pressure and a slowing of pulse rate. Although the negative inotropic action of atabrine has been utilized in attempting to produce heart failure experimentally (20), to our knowledge quinidine has not been employed in this manner. In our experiments, quini-

TABLE 1. BILATERAL RISES IN AURICULAR PRESSURE (MM. OF WATER)

CAT	RIGHT AURICLE			LEFT AURICLE		
	Control	Quinidine	Change	Control	Quinidine	Change
A. With Near Lethal Doses of Quinidine						
3	19	51	32	78	100	22
5	25	49	24	20	38	18
7	30	60	30	69	110	41
9	32	88	56	81	103	22
Mean	26	62	36	62	88	26
CAT	CONTROL	AUR. FIB.	CHANGE	CONTROL	AUR. FIB.	CHANGE
B. With Auricular Fibrillation						
8	20	60	40	39	110	71
9	40	62	22	108	152	44
12	28	40	12	119	130	11
Mean	29	54	25	89	131	42

dine given intravenously in near lethal doses of 15 to 30 mg/kg. of body weight produced a severe arterial pressure drop of 30 to 80 mm. Hg, a bradycardia, a pulse pressure increase, cardiac dilatation and an elevation of auricular pressure, usually bilateral. Table 1 *A* shows the bilateral rises in auricular pressure of 4 hypervolemic cats given 25 mg/kg. of quinidine. Right auricular pressure rose an average of 36 mm. of water and left auricular pressure, 26 mm. Similar results were obtained in cats with normal blood volumes. Spontaneous recovery from the cardiotoxic action of quinidine was attended by a gradual return of arterial pressure, heart rate and cardiac size to control levels and by a bilateral drop of auricular pressure. Although complete recovery takes 2 to 3 hours (16), auricular pressures returned to prequinidine levels in 3 to 15 minutes. It is therefore apparent that severe weakening of the myocardium in normal and plethoric cats produces only moderate elevations of auricular pressure.
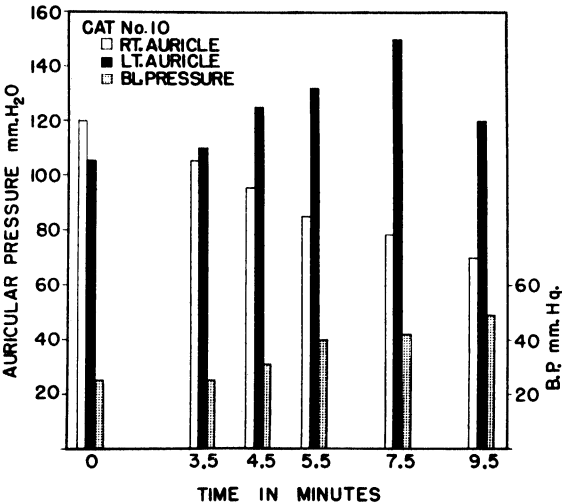
Unilateral Elevations of Auricular Pressure with Quinidine. In the same cat mani-

festing bilateral rises in auricular pressure after further infusion, a repetition of the
near lethal dose of quinidine produced a unilateral elevation of auricular pressure.
This consisted of an elevation of right auricular pressure and a drop in left. Table

TABLE 2. UNILATERAL RISES IN AURICULAR PRESSURE (MM. OF WATER)
Same cats as in table 1 with greater blood volumes

CAT	RIGHT AURICLE			LEFT AURICLE		
	Control	Quinidine	Change	Control	Quinidine	Change
A. With Near Lethal Doses of Quinidine						
3	55	90	35	112	89	−23
5	20	78	58	95	70	−25
7	110	240	130	260	195	−65
9	90	115	25	150	130	−20
Mean	69	131	62	154	121	−33
CAT	CONTROL	AUR. FIB.	CHANGE	CONTROL	AUR. FIB.	CHANGE
B. With Auricular Fibrillation						
8	62	80	18	155	124	−31
9	63	90	27	190	115	−75
12	50	62	12	164	140	−24
Mean	58	77	19	169	126	−43

Fig. 2. SPONTANEOUS RECOVERY of heart weakened by quinidine. At zero time maximal depression of the heart with quinidine is represented by elevated auricular pressures and a low arterial pressure. Note that the first $7\frac{1}{2}$ min. of early recovery are attended by a rising left auricular pressure as right auricular pressure declines and arterial pressure rises. Finally left auricular pressure begins to fall as arterial pressure continues to rise.

2 *A* lists the values obtained during these unilateral rises. Average rise in the right
auricle was 62 mm. of water, while average drop in the left auricle was 33 mm.

The opposite type of unilateral auricular pressure elevation may also occur but
only during the phase of early recovery from quinidine. *Cat 10* (fig. 2) is a representative experiment of this type, showing an elevation of left auricular pressure
and a decline in right auricular pressure in the recovery period after a near lethal

dose of quinidine. As recovery continued both auricular pressures gradually dropped to control levels.

The unilateral right auricular pressure rise during quinidine administration was attended by a more marked dilation of the right ventricle indicating predominantly rightsided weakness. Similarly in the unilateral left auricular pressure rise occurring during early recovery from quinidine the left ventricle was observed to be relatively more dilated than the right, proof that the right heart was recovering before the left.

Effect of Auricular Fibrillation on Auricular Pressures. Auricular fibrillation is also known to weaken ventricular contraction (5). The bilateral and unilateral right auricular pressure responses obtained during quinidine-induced myocardial
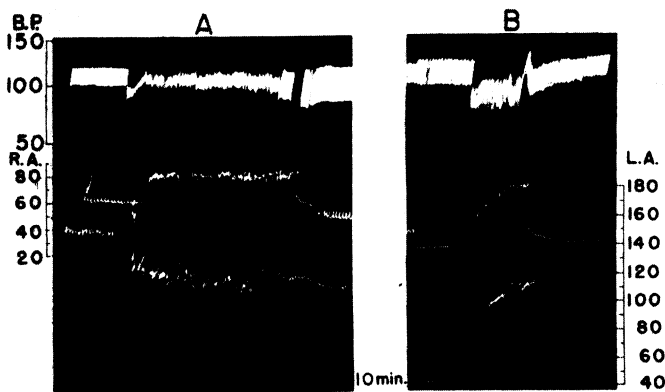

Fig. 3. UNILATERAL AND BILATERAL AURICULAR PRESSURE RISE of myocardial weakness. Reading from top to bottom: blood pressure (B.P.) in mm. Hg; time in seconds; right auricular pressure (R.A.) and left auricular pressure (L.A.) in mm. of water. The irregularity in the arterial pressure tracing indicates the period of electrically induced auricular fibrillation. *A:* Unilateral elevation of right auricular pressure with a drop in left auricular pressure in a hypervolemic cat immediately after blood volume increased by infusion. *B:* Bilateral elevation of auricular pressures in the same cat 10 min. later. Note that resting auricular pressures are significantly lower in *B* as compared to *A.*

weakness were duplicated with auricular fibrillation (fig. 3). Thus two methods of inducing cardiac weakness produced qualitatively similar results. Auricular pressures for the bilateral rise with auricular fibrillation are given in table 1 *B*, while pressures during the unilateral rise in the same three animals are tabulated in table 2 *B*. It is to be noted that in the unilateral response elicited with both quinidine and auricular fibrillation, the resting auricular pressures were at a higher level than those of the bilateral response.

Effect of Epinephrine and Pituitrin on Auricular Pressure. It has been noted above that recovery of the heart weakened by quinidine may be attended by a rising left auricular pressure. In an attempt to elucidate this mechanism epinephrine, because of its positive inotropic action, was administered to the weakened heart and its effect on left auricular pressure noted.

Epinephrine in an intravenous dose of 20 to 40 gamma, administered to both nor-

mal and hypervolemic cats, caused a moderate and transient elevation of left auricular pressure, 30 to 71 mm. of water, lasting 1 to 9 minutes. There was no significant change in right auricular pressure. This was in sharp contrast to the marked, prolonged elevation of left auricular pressure produced by the same amounts of epinephrine in the hypervolemic cat whose heart had been weakened by quinidine. This effect is illustrated in figure 4 *A*, in which left auricular pressure rose 177 mm. and 165 mm. of water in *cats 1* and *10* respectively. Return to control levels took 28 and 47 minutes. The rise of right auricular pressure in *cat 1* was only 30 mm. water. In *cat 10* right auricular pressure dropped 44 mm. as left rose to 165.

In order to determine whether or not this left auricular pressure rise occurring in the weakened heart was a result of peripheral vasoconstriction or the direct stimulant action of epinephrine on the heart, posterior pituitary extract was used. With the use of posterior pituitary principles rises in left auricular pressure can only be attributed to peripheral vasoconstriction since cardiac performance is not enhanced

Fig. 4. EFFECT OF EPINEPHRINE AND PITUITRIN on left auricular pressure of the weakened heart. *A:* At zero time 20 and 40 gamma of epinephrune intravenously to *cats 1* and *10*, respectively. *B:* At zero time 2 IU of pituitrin intravenously to *cats 10* and *12* respectively. Note elevation of left auricular pressure is neither of comparable degree nor duration to that produced by epinephrine.
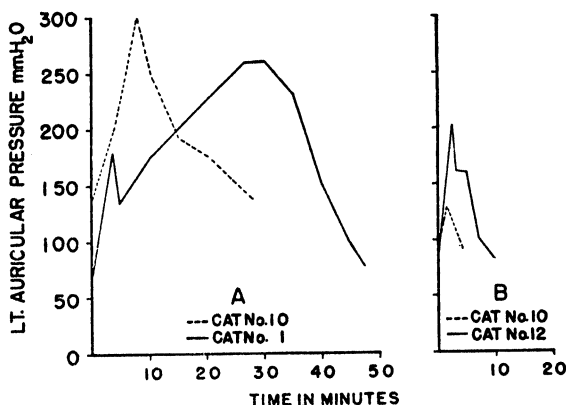
but on the contrary further depressed because of coronary vasoconstriction (7). Contrasted to epinephrine, pituitrin administered to the weakened heart had far less effect on left auricular pressure. In figure 4 *B* it is to be noted that two IU of posterior pituitary extract produced only moderate and transient increases in left auricular pressure: 38 mm. in *cat 10* and 128 mm. in *cat 12*. Return to control levels occurred in 4 and 10 minutes. Right auricular pressure dropped very slightly in *cat 10*, i.e. 8 mm., and was unchanged in *cat 12*. Thus it is clear that the magnitude and duration of left auricular pressure rise is much greater with 20 to 40 gamma of epinephrine than it is with two IU of pituitrin.

The positive inotropic action of epinephrine on left auricular pressure could only be ascertained by demonstrating that the degree of vasoconstriction produced with epinephrine and pituitrin in the amounts used was equal. Utilizing changes in mean arterial blood pressure as an index of the degree of vasoconstriction, it was observed that pituitrin produced at least an equal if not greater peripheral vasoconstriction than epinephrine. Thus 20 gamma of epinephrine in *cat 1* produced no elevation of arterial pressure as left auricular pressure rose 177 mm. of water.

In cat 10, 40 gamma of epinephrine elevated mean arterial blood pressure from 28 to 40 mm. Hg. However, arterial pressure had returned to control levels when the peak left auricular pressure rise occurred 7 minutes later. Pituitrin in a dose of 2 IU in the same cat caused a 10-mm. rise of blood pressure (40–50 mm. Hg) which persisted throughout the left auricular pressure rise and even after it had returned to control levels. In cat 12, 2 IU pituitrin elevated blood pressure 55 mm. Hg (25–80 mm.) at the time of peak left auricular pressure rise. Blood pressure continued to rise (90 mm.) five minutes after pituitrin was given and while left auricular pressure was dropping. When left auricular pressure had returned to control levels, the arterial pressure was still 50 mm. Hg above its pre-pituitrin value. These results indicate that although the peripheral vasoconstriction produced by epinephrine contributes in part to the unilateral left auricular pressure rise, its direct stimulant action on the weakened heart is the primary factor.

DISCUSSION

The inequality of myocardial weakness and recovery of the right and left hearts that appears manifest in the unilateral elevations of right and left auricular pressure lends itself to interpretation in terms of the volume-tension curve of cardiac muscle as promulgated by Frank (21), Kozowa (22), Starling (23), and others, (24, 25). In the previous paper it has been pointed out the heart may be considered to be a hollow elastic sphere. Increased venous return causes a distension of the cardiac chambers because of the higher distending pressure. At this increased heart muscle fiber length the mechanical advantage is decreased (27, 28). As a result of this the high venous distending pressure is not only maintained at a high level but may be actually increased because of the inability of the heart chamber to empty itself completely. Thus the heart is working at a high level of the volume-tension curve the events of which are summarized as follows: Changes in initial length change the mechanical advantage at which the heart-muscle fiber performs (28). The corollary of such an interpretation is that a high venous pressure is not the cause of distention of the heart chambers but rather that a high venous pressure results from such distention, i.e. an inability of the heart to empty completely. When a heart works on a higher level of this curve four events have occurred: 1) cardiac radius is increased; 2) there is decreased shortening of the myocardium during systole; 3) there is increased residual blood in the heart; and 4) auricular pressure is elevated.

In the experimental animal the heart can be made to ascend the curve by increasing venous return so that cardiac radius is increased. This can be accomplished by infusion or by decreasing cardiac output with quinidine or auricular fibrillation, which in effect increases venous return. Thus, during the bilateral elevation of auricular pressure with quinidine or auricular fibrillation the reduction in cardiac output on both sides of the heart amounts to an increased venous return. This causes both sides of the heart to work on a higher level of the curve and hence the elevated auricular pressures. The reverse situation prevails during the bilateral decline of auricular pressure accompanying recovery of the heart from quinidine.

Under certain conditions, particularly when the blood volume is very large and

the heart very weak, it has been shown that unilateral elevations of auricular pressure may occur. Assuming that the curtailment of cardiac output is equal on both sides of the heart, unilateral elevations of right auricular pressure with quinidine and fibrillation appear to indicate that the diastolic size of the right heart becomes disproportionately greater than the left. This was observed visually in our experiments; i.e., whenever right auricular pressure was greater than the left, the right ventricle was markedly dilated compared to the left. During such a state of affairs the right heart is working on a higher level of the volume elasticity curve and the left heart on a lower level. Under these conditions the right ventricle has a smaller coefficient of elasticity than the left ventricle but may maintain an output equal to the left ventricle because it is at a more advantageous volume/surface area position.

This interpretation is substantiated by the fact that the heart was always on a higher level of the volume elasticity curve prior to the unilateral auricular pressure response of myocardial weakness, contrasted to the bilateral auricular pressure response in the same animal (see mean control, auricular pressure, tables 1 and 2).

The unilateral elevation of left auricular pressure during the recovery from quinidine implies that the right heart is recovering more rapidly than the left, e.g. that the right heart is descending the volume-elasticity curve more rapidly than the left or that its cardiac radius is more rapidly approaching normal limits than that of the left heart. That the right heart may recover before the left has been suggested by Richards *et al.* (26). They observed that the right auricular pressure dropped as the vital capacity continued to decrease in a patient with heart failure following an infusion.

Patterson and Starling suggested that under certain conditions one side of the heart could fail before the other (27). They interpreted differences in the degree of rise of right and left auricular pressure in the heart-lung preparation as indicating such a situation. In this investigation it has been shown that auricular pressures can change in opposite directions. This would appear to furnish evidence that the dynamics of the right and left heart do dissociate. Finally, none of our observations support the contention of Henderson and Prince that the rise of right auricular pressure attending pulmonary engorgement is caused by a decreased capacity of the right ventricle as a result of displacement of the intraventricular septum during diastole (29). In our series of experiments dilation of the right auricle and ventricle always accompanied a unilateral rise of right auricular pressure.

SUMMARY

Acute heart failure was produced in open-chest cats by massive infusion with saline followed by sub-lethal doses of quinidine. This was usually attended by elevation of both right and left auricular pressures. In the same cat, further infusion and a second dose of quinidine was attended by a rise of right auricular pressure and a drop in the left auricular pressure. These results were duplicated with auricular fibrillation following massive infusion, thus showing that it is not a specific drug effect but that it is weakness of the myocardium that produces the changes in auricular pressure. As the animal recovered from the quinidine usually both right and left auricular pressure slowly declined to normal values. In the cats whose

blood volume had been greatly increased the left auricular pressure rose and the right auricular pressure fell in the quinidine recovery phase.

Epinephrine was used to accentuate the recovery phase in the unilateral left auricular pressure rise attending recovery from quinidine. Proof was advanced that it was largely the positive inotropic action of epinephrine on the heart rather than the system vasoconstriction which caused the left auricular pressure rise. This was accomplished by giving an equivalent dose of pituitrin which has purely vasoconstrictive properties.

The results permit the conclusion that in the presence of marked hypervolemia the onset of severe myocardial weakness and the recovery from that weakness may produce dissociation of the dynamics of the right and left ventricle. This phenomenon is simply explained by the observation that in this dissociation one ventricle is markedly dilated as compared to the other. Hence the deduction that under appropriate conditions one ventricle may function at a different level of Starling's Curve than the other.

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HEAT LABILITY OF A DEPRESSOR SUBSTANCE PRESENT IN HUMAN URINE: EFFECTS OF SECTION OF VAGUS NERVES, LIGATION OF CAROTID ARTERY AND OF AUTONOMIC BLOCKADE UPON DEPRESSOR RESPONSE[1]

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IT HAS been reported previously that a decrease in the mean arterial pressure, a decrease in the peripheral resistance and an increase in the cardiac output follow the intravenous injection of urine into dogs (1). A method of assay for the depressor effect of urine is presented here, together with our analysis of the effects of heating the urine, the effects of vagus nerve section and of autonomic blockade upon the depressor response.

METHODS

Mongrel dogs weighing 5 to 10.5 kg. were anesthetized with either pentobarbital sodium, 30 mg/kg., or amytal sodium, 50 mg/kg. One femoral artery was cannulated for recording mean arterial pressure using a mercury manometer, and one femoral vein was cannulated for making the injections. Unless otherwise indicated, all tests were performed on such animals. In a few dogs, the vagi were sectioned and in others the carotid arteries were compressed.

The various solutions were injected by syringe at a constant rate. Each injection, timed by an audible one per-second signal, took 20 seconds for completion. The injections were made at intervals of not less than 10 minutes, and before a subsequent injection was made the mean arterial pressure (MAP) had returned to the control value and had remained there for several minutes.

All urine samples were collected from healthy young men. The samples were filtered through filter paper if necessary. Immediately after collection they were placed in cellophane bags and dialyzed against running tap water for 24 hours. The bags were then transferred to distilled water which was usually changed four to six times during another 24-hour period. The dialysis was carried out in a refrigerator at an average temperature of 9° C. and all samples were frozen and stored in the low-temperature refrigerator until used.

Just prior to using, some of the dialyzed urine samples were heated in a constant temperature water bath at 40°, 60° and 80° C. for 120 minutes; some samples were heated in a boiling water bath for various time periods. All urine samples were adjusted to the original volume after heating by the addition of distilled water.

Some dialyzed urine samples were concentrated by vacuum distillation at a temperature of 38° to 41° C. The distillate was saved for injection, and the residue was injected as such, or it was diluted to the original volume by the addition of distilled water. Histamine hydrochloride, 5 μg/cc., was injected in doses of 2 to 6 cc. in order to compare the depressor effect of urine with that of histamine.

RESULTS

Typical Response. Typical mean arterial pressure (MAP) responses to urine and histamine are reproduced in figure 1. They are from the same animal and were chosen to illustrate responses which we considered to be equivalent. In this instance 5 cc. of urine was equivalent to 10 μg. of histamine.
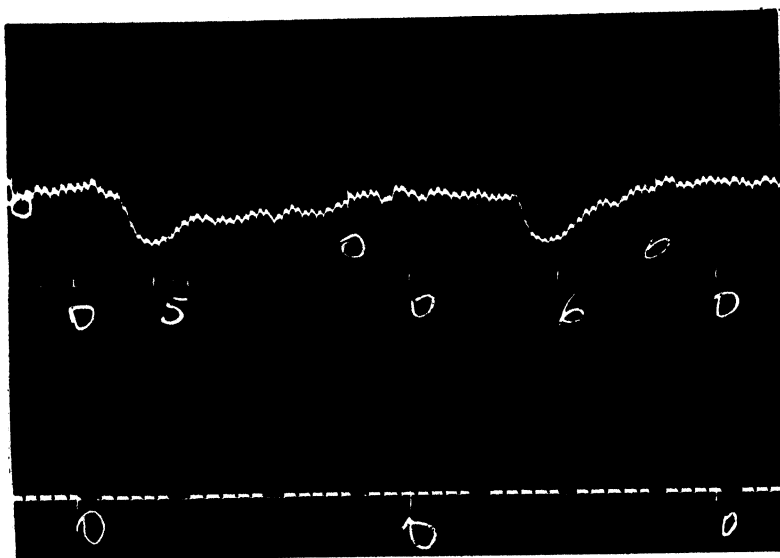
Fig. 1. COMPARISON OF DECLINE in mean arterial pressure in response to 5 cc. dialyzed urine (5) and to 10 μg. histamine (6) injected intravenously into an 8-kg.dog anesthetized with sodium amytal (*exp. N-31*).

Method for Expression of Response. Possible methods for expressing responses of this type include *a*) maximum change in mean arterial pressure (−ΔMAP), *b*) duration of response (sec.), *c*) response at some fixed interval after the injection and *d*) the integrated response (MAP·seconds), i.e., the area bounded by the base line and the MAP curve which results from the injection. For these studies we have used the maximum change in MAP. This may be expressed as absolute (mm) decline in mean arterial pressure (−ΔMAP) or as percentage decline (% −ΔMAP

$= \dfrac{\text{control MAP} - \text{experimental MAP}}{\text{control MAP}} \times 100$). In figure 2 and table 1 we have

analysed the response in 83 sets of injections using both methods of expression. Each set represented 2 or more injections of the same amount of substance (urine or histamine) into the same dog.

Reproducibility of Response. The maximum variability of response within each set of injections of urine and histamine in both intact and vagotomized dogs with respect to the mean response will be found in figure 2. It will be seen that the maximum variability increases with increasing mean response. However, most of the values for maximum variability lie below the line representing ±60 per cent of the mean response. Greater responses and greater variability were found in the vagotomized animals. However, with the exception of histamine in the vagoto-
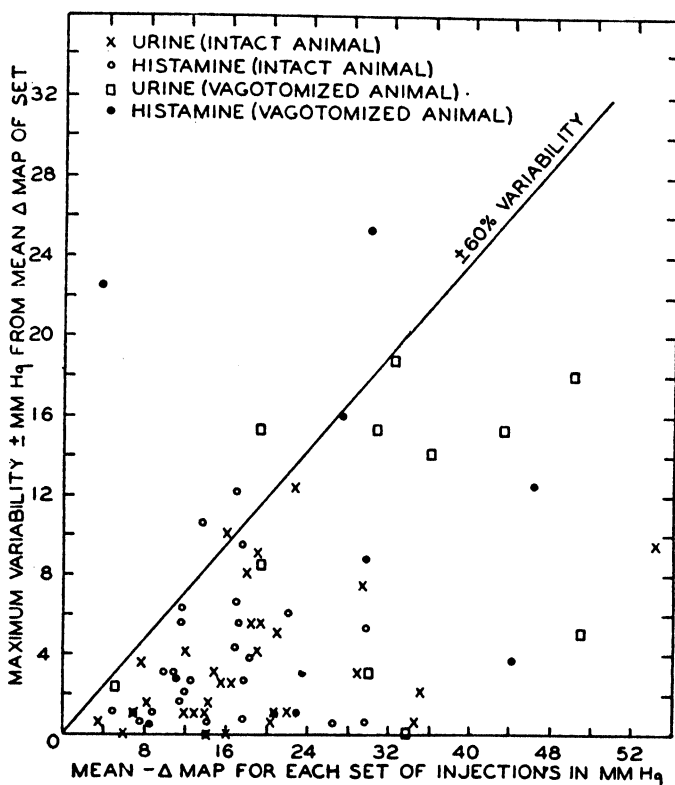
Fig. 2. RELATION between maximum variability within each set of injections and mean response within each set of injections. Each point represents the greatest single deviation from the mean response within each set.

mized animals, there was no difference in the percentage maximum variability of the substances injected in the two animal preparations.

An analysis of the reproducibility of the decrease in mean arterial pressure $(-\Delta MAP; \%-\Delta MAP)$ with repeated injections of the same dose of the same sample in the same dog is illustrated by the data in table 1. In almost 100 per cent of the sets of data in the intact dog the maximum deviation of any one response within a set did not differ from the mean $-\Delta MAP$ of the set by more than ±12 mm (table 1, *I*), and 68 to 72 per cent were within ±4 mm (table 1, *columns A* and *C*). In the dogs with vagi sectioned (*columns B* and *D*) the variability was

greater. When the maximum variability of an individual determination in the intact dog was expressed as percentage of the mean response of the set approximately 60 per cent of the sets showed a maximum variation of less than ±20 per

TABLE 1. VARIABILITY OF DATA

Percentage of total sets in each group

| | HISTAMINE | | | | URINE | | | |
| | Intact Dog | | Vagi Sectioned | | Intact Dog | | Vagi Sectioned | |
	$-\Delta$ MAP	$\frac{-\Delta \text{MAP}}{\text{MAP}} \times 100$	$-\Delta$ MAP	$\frac{-\Delta \text{MAP}}{\text{MAP}} \times 100$	$-\Delta$ MAP	$\frac{-\Delta \text{MAP}}{\text{MAP}} \times 100$	$-\Delta$ MAP	$\frac{-\Delta \text{MAP}}{\text{MAP}} \times 100$
Deviation of individual data about mean for set	**A**		**B**		**C**		**D**	
±0 to ±4 mm. Hg	67.8%		54.5%		71.8%		25.0%	
±4.1 to ±8 mm. Hg	21.4%		0 %		15.6%		8.3%	
±8.1 to ±12 mm. Hg	10.7%		9.1%		9.4%		8.3%	
±12.1 to ±16 mm. Hg	0 %		18.2%		3.1%		41.6%	
±16.1 to ±20 mm. Hg	0 %		9.1%		0 %		16.7%	
±20.1 to ±24 mm. Hg	0 %		0 %		0 %		0 %	
±24.1 to ±28 mm. Hg	0 %		9.1%		0 %		0 %	
I	99.9%		100.0%		99.9%		98.9%	
Number of sets in group	28		11		32		12	
Average of maximum deviation in each set from mean $-\Delta$MAP of each set	3.3 mm. Hg		8.8 mm. Hg		3.4 mm. Hg		10.9 mm. Hg	
mean of $-\Delta$MAP of all sets in each group	23.0 mm. Hg		29.3 mm. Hg		18.9 mm. Hg		32.0 mm. Hg	
Deviation of individual data about mean for set	**E**	**F**	**G**	**H**	**J**	**K**	**L**	**M**
±0 to ±20%	57.1%	60.7%	45.5%	54.6%	65.6%	56.3%	25.0%	16.7%
±20.1 to ±40%	28.6%	21.4%	36.1%	18.2%	18.8%	28.1%	33.3%	58.3%
II ±40.1 to ±60%	3.6%	3.6%	9.1%	18.2%	15.6%	12.5%	25.0%	25.0%
±60.1 to ±80%	10.7%	10.7%	9.1%	0 %	0 %	3.1%	8.3%	0 %
±80.1 to ±100%	0 %	3.6%	0 %	9.1%	0 %	0 %	8.3%	0 %
	100.0%	100.0%	99.8%	100.1%	100.0%	100.0%	99.9%	100.0%
Number of sets in group	28	28	11	11	32	32	12	12

$-\Delta$ MAP = decline in mean arterial pressure expressed as mm. Hg.

$\frac{-\Delta \text{MAP}}{\text{MAP}} \times 100$ = decline in mean arterial pressure expressed as % of control mean arterial pressure.

A–D = percentage of total sets in which the maximum deviation of an individual determination differed from the mean of the set by the number of mm. Hg indicated in the left-hand column.

E–M = percentage of total sets in which maximum deviation of an individual determination differed from the mean of the set by the percentile amount indicated in the left-hand column.

cent of the mean $-\Delta$MAP (table 1, *II*) and, particularly with urine, 100 per cent of the sets showed a maximum variation less than ±60 per cent of the mean $-\Delta$MAP for the set (table 1, *columns E and J* and fig. 2). Expressed in this way the variability was still slightly greater in the vagotomized than in the normal dogs (table 1, *columns F and K*). Since one standard deviation includes 68 per cent of the

observations we might, thus, without actual computation, consider the standard deviation to be between ±20 and ±30 per cent of the mean −ΔMAP.

The mean arterial pressure varied from time to time in the experiments. Since the response to a given injection might be expected to be different at high as compared with low control mean arterial pressure we also calculated the percentage change in mean arterial pressure (%−ΔMAP) for each injection. As shown in *columns F, H, K* and *M* in table 1, the variability was no less with this mode of expression than when the response was expressed simply as maximum decrease in MAP recorded in mm. Hg. Since no decrease in variability of response was obtained by vagus section or by expressing the results as %−ΔMAP, the results in the remainder of the paper are expressed as −ΔMAP (in mm. Hg) obtained on intact anesthetized dogs.
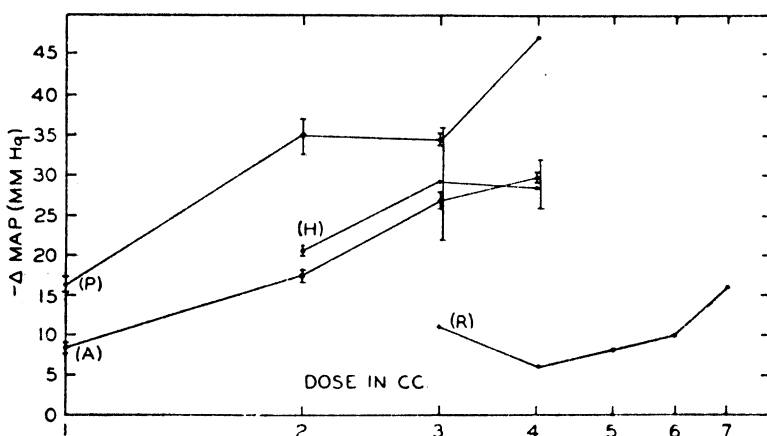

Fig. 3. COMPARISON of the log-dose and the average response with histamine and dialyzed urine in the same dog. *A:* histamine, 1 cc. = 5 μg., 3 injections each dose; *P:* unheated urine, 2 injections each dose; *H:* urine heated in a boiling water bath for 30 min., 2 injections each dose; *R:* urine refluxed for 1 hr., 1 injection. *Bar:* maximum range. The same original urine sample was used in all injections. −ΔMAP = decrease in mean arterial pressure in mm. Hg following injection. Anesthesia: amytal sodium (*Exp. N-12*).

From the above data it would appear that a response to a single injection would have to differ from the mean of a set of 2 or more injections by more than ±60 per cent (2 standard deviations) to be significant and the mean −ΔMAP of a set of 3 injections would have to differ from the mean of another set of 3 injections by ±50 per cent to be significant ($\sqrt{1/3 + 1/3} \times 60\% = 50\%$).

Dose-response Relationship. In 15 series of injections of histamine and urine into 12 dogs we explored the dose-response relationship. The response was expressed as −ΔMAP in mm. Hg. The data were plotted on regular, on double log and single log paper. In no instance were consistently straight lines obtained. The most commonly obtained dose-response relationship was comparable to that in figure 3. According to these curves a 50 per cent increase in response would require doubling the dose and a 50 per cent decrease in response would be expected by halving the dose. On the basis of this data plus that in section 3 (*above*) it would appear that

the accuracy of this method of assay ($\pm 2 \times$ standard deviation) would be $+100$ to -50 per cent of the apparent concentration (or dose).

As shown in figure 3 there was usually a clear-cut shift in the locus of the plots upon heat treatment et cetera. Because of the variability of the plots we elected, insofar as possible, in making comparative assays to adjust the doses of the substances so that the same response was obtained with each. Comparison was then made on the basis of the required doses (fig. 4).

Effect of Section of Vagus Nerves and of Autonomic Blockade upon Depressor Response to Urine. The effects of bilateral vagal section, bilateral carotid artery ligation, the intravenous injection of 200 mg. of tetraethyl ammonium chloride (TEAC)[2], and the intravenous injection of 10 mg. of Priscol (benzazoline hydrochloride, 2 benzylimidazoline hydrochloride)[3] upon the depressor effect of urine will be found in
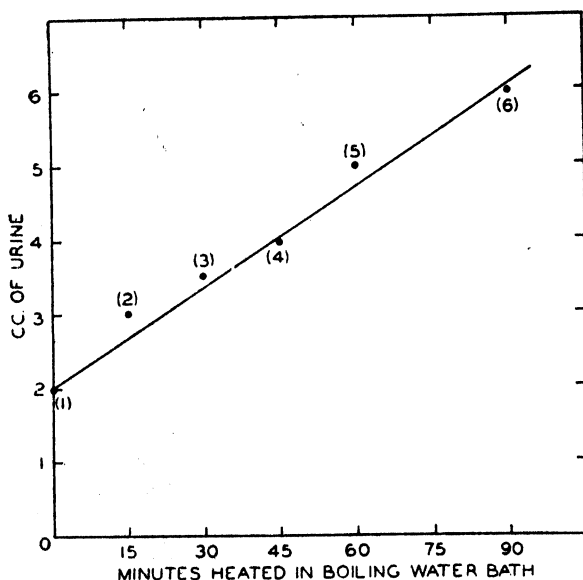

Fig. 4. VOLUME of dialyzed urine heated in a boiling water bath necessary to produce a decrease of approximately 13 mm. Hg in the mean arterial pressure of anesthetized dog. Anesthesia: amytal sodium. The same original urine sample was used throughout. Actual $-\Delta$MAP: (*1*) 13, (*2*) 16, (*3*) 14, (*4*) 13, (*5*) 17, (*6*) 13 mm. Hg.

table 2. It will be seen, in the last column, that vagal section increased the average urine depressor effect approximately 80 per cent, while subsequent bilateral carotid artery ligation with hypertension returned the urine depressor effect to approximately the control value. The injection of TEAC diminished the hypertension produced by carotid artery ligation and increased the depressor effect of the urine. The injection of Priscol increased the average mean arterial pressure, but, in contrast to carotid artery ligation, it caused a marked increase in the depressor effect of urine.

Effects of Heating the Urine upon the Depressor Response. a) *Effect of duration of period of heating.* The effects of heating the urine samples on the depressor response are presented in figures 3 and 4 and in table 3. Figure 3 shows the effects on the

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[2] Supplied by Parke, Davis and Co., Detroit, Mich.
[3] Supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

depressor response produced by heating dialyzed urine in a boiling water bath for periods of 15, 30, 45, 60 and 90 minutes. The volume of urine necessary to give a $-\Delta$MAP of approximately 13 mm. Hg was determined for the control and for each of the heated urines. This volume was plotted against the time the urine was heated. It will be seen that a straight line function results.

TABLE 2. EFFECT OF VAGAL SECTION, CAROTID ARTERY LIGATION, INJECTION OF 200 MG. OF TETRA-ETHYLAMMONIUM CHLORIDE (TEAC), AND INJECTION OF 10 MG. OF PRISCOL ON THE HYPOTENSIVE RESPONSE TO THE INJECTION OF POOLED FRESHLY-VOIDED (NON-DIALYZED) URINE
Exp. N-2. Pentobarbital Sodium Anesthesia

SUCCESSIVE PROCEDURES	NO. OF INJEC-TIONS	VOL. URINE INJECTED RANGE IN CC.	PRE-INJECTION MAP		$-\Delta$MAP[1] RANGE	$-\Delta$MAP/CC. URINE	
			Range	Average		Range	Average
			mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
Control.....................	9	4–6	154–168	161	10–18	1.7–4.5	2.8
Both vagi sectioned..........	4	6	159–172	164	27–32	4.5–5.3	5.0
Both carotids ligated........	8	6–8	188–208	199	10–32	1.3–4.0	2.5
TEAC......................	4	4–6	100–179	130	17–44	2.8–7.3	4.6
Priscol....................	2	4–6	173–185	179	28–64	7.0–10.6	8.8

[1] $-\Delta$MAP = decrease in mean arterial pressure.

TABLE 3. EFFECT OF HEATING DIALYZED URINE ON THE HYPOTENSIVE RESPONSE TO ITS INJECTION
Exp. N-14. Amytal Sodium Anesthesia

URINE NO.	HEATING		NO. OF INJECTIONS	VOL. INJECTED RANGE	$-\Delta$MAP[1] RANGE	CC./15 MM. HG $-\Delta$ MAP	
	Temp.	Time				Range	Average
	°C.	*min.*		*cc.*	*mm.Hg*		
13A	o	o	9	1–4	7–28	1.4–2.5	1.9
13B	40	120	2	3–3.5	18–20	2.5–2.7	2.6
13C	60	120	4	3–3.5	16–22	2.1–2.8	2.5
13D	80	120	3	3–4	16–24	2.5–2.8	2.7
13E	B.W.[2]	30	8	2–6	8–19	2.1–6.3	3.8
13F	B.W.	60	3	6–6.5	10–26	3.8–10.0	6.6
13G	B.W.	90	3	9–10	14	7.2–9.4	7.9
13H	B.W.	120	3	12–14	10–24	5.9–18.0	13.5

[1] $-\Delta$MAP = fall in mean arterial pressure.   [2] B.W. = boiling water bath.

b) Effect of boiling water bath as compared with refluxing. While the relationship between log-dose and the response ($-\Delta$MAP) in figure 1 is not strictly a straight-line function for either histamine or the urine samples, it is clear that when urine, heated in a boiling water bath for 30 minutes (*H*) or refluxed for 1 hour (*R*), is compared with unheated urine (*P*) there is a decrease in the depressor response. Put another way, an increased dose of (*H*) or (*R*) is required to produce the same response as that obtained with (*P*). It is to be noted that the greatest destruction of the depressor factor was produced by the reflux condensor treatment.

c) Effect of temperature at which urine is heated. The last column in table 3 shows that the depressor effect of urine is decreased from the control effect by heating for

120 minutes at 80° C. or below, but the temperature of heating within this range appears to be immaterial. However, the decrease in depressor effect of urine when it is heated in a boiling water bath is dependent upon the duration of heating.

   d) Effect of vacuum distillation. Table 4 shows that when dialyzed urine is distilled *in vacuo* the depressor substance remains in the residue almost entirely. This is illustrated by comparing 17 P (unheated) with 17 RD (residue diluted to the original volume with distilled water). There appears to be little or no decrease in the depressor effect as a result of this procedure.

TABLE 4.   EFFECT OF VACUUM DISTILLATION OF URINE ON THE HYPOTENSIVE RESPONSE TO ITS INJECTION

Amytal Sodium Anesthesia. Distillation Temperature, 38–41° C.

EXP. NO.	URINE NO.[1]	NO. OF INJECTIONS	VOL. INJECTED RANGE	−ΔMAP[2] RANGE	CC./15 MM. HG−ΔMAP	
					Range	Average
			cc.	mm. Hg		
N-18	17P	4	0.3–0.5	7–20	0.38– 0.65	0.57
	17RD	1	0.5	13		0.58
	17D	3	5–20	5–10	15.0 –30.0	20.6
N-24	17P	4	1–1.5	9–18	1.3 – 2.5	1.8
	17D	2	30	11–15	30.0 –37.5	33.7
	17R[3]	5	0.2–1	10–38	0.22– 0.40	0.31
	17RD	2	1.5	14–20	1.1 – 1.6	1.4
N-25	21P	2	2	11–21	1.4 – 2.7	2.1
	21RD[4]	2	2–3	10–13	3.0 – 3.5	3.3
	21D	5	7–10	15–29	5.0 – 8.8	6.0

[1] P = unheated dialyzed; D = distillate; R = residue from distillate; RD = residue diluted to original volume with distilled water.   [2] −ΔMAP = fall in mean arterial pressure.   [3] Concentrated 3.45 times.   [4] Distilled to dryness, dissolved in 25 cc. distilled water: concentrated 20 times.

DISCUSSION

   A depressor substance, callicrein or padutin, present in urine, pancreatic juice and intestinal secretions has been described by Frey and co-workers. This material is heat labile. Werle (3) has reported that callicrein obtained by pancreatic fistula in the dog is reduced in activity 85 to 95 per cent when the preparation is heated for 3 minutes over an open flame. Westerfeld *et al.* (4) reported that heating a callicrein preparation in a boiling water bath for 20 to 30 minutes markedly diminished but never completely abolished the hypotensive effect. Wollheim (5) reported that callicrein was destroyed in 10 minutes when heated at 100° C. in neutral, acid or alkaline media.

   In table 3 it will be seen that heating dialyzed urine for 120 minutes at 40, 60 and 80° C. diminished the depressor effect of the urine approximately 30 per cent, and that the decrease in depressor effect was not related to the temperature at which the urine was heated. This change is within the apparent limits of error of the method. In contrast, heating the urine for 30 minutes in a boiling water bath decreased the depressor effect approximately 55 per cent. The decrease in depressor effect increased as the duration of heating at this temperature was prolonged, i.e.

to 13 per cent of the control after 2 hours. This change is significant. We interpret these data as evidence for two depressor substances present in urine. One of these is quite heat labile, being destroyed at temperatures below 100° C. and is probably callicrein, while the other substance, although still heat labile at 100° C., is much more resistant to heating than is callicrein. This interpretation is strengthened by the data in figure 2 where refluxing the urine sample for one hour did not completely destroy the depressor activity, although it was less than the control activity or the activity of urine heated in boiling water for 30 minutes.

Wollheim (5) has previously reported an urinary depressor substance, which he called depressan. He found that this substance was active after heating at 100° C. for 10 minutes in a neutral solution, was active after 5 minutes heating in acid solution, but was destroyed after 30 seconds heating in alkaline solution. It is quite probable that the depressor substance reported here is very similar to if not identical with the depressor substance of Wollheim.

From the data given in table 2, it would appear that the site of action of the depressor substance reported here is primarily on the peripheral vessels. When a vasoconstriction was elicited by bilateral carotid artery ligation the depressor effect was diminished by 50 per cent from the previous value obtained after bilateral vagal section. However, when TEAC, which has been shown by Acheson and Pereira (6) to block autonomic ganglia, was injected the depressor effect of the urine returned to approximately the value found before carotid artery ligation. When Priscol, which is reported by Chess and Yonkman (7) to be sympatholytic and adrenolytic in reference to the sympathetic neural control of blood pressure, was injected it caused a marked increase in the depressor effect of the urine. This may be due to the more complete abolition of tonic vasoconstriction following Priscol, or to a reversal of effect of another substance with constrictor properties, i.e., an epinephrine-like substance.

The possible relationship between this depressor substance and the problem of hypertension is obvious. Hypertension may be the result not only of an excess quantity of angiotonin or hypertensin (as has been stressed by much recent work), but a decrease in the amount of the urinary depressor substance may also contribute to the elevation of arterial blood pressure. In essential hypertension it is possible that there is a decrease in the depressor substance, which might lead to an imbalance in the regulation of peripheral resistance. Wollheim (5) has reported that his depressan is either entirely missing or at least markedly decreased in the urine of patients with essential hypertension. On the other hand, patients who had a primary renal lesion and also had hypertension did not show any significant alteration in the amount of depressan excreted in the urine. The site of formation of the urinary depressor substance reported here is not known nor is it known whether or not the substance is present in blood.

SUMMARY

The depressor effect of urine was studied by recording the decrease in mean arterial pressure ($-\Delta$MAP) produced by intravenous injection of the urine into anesthetized dogs. Analysis of results suggests that the mean of the response to a set

of three injections must differ by ±50 per cent from that of another set to be significant, and that the apparent concentration of a dilator substance estimated by this method may differ from the true concentration by −50 per cent to +100 per cent.

Evidence is presented that, in addition to callicrein, there is present in normal dialyzed human urine a substance which causes a decrease in the mean arterial pressure of dogs anesthetized with amytal sodium when the urine sample is injected intravenously. This depressor substance is somewhat heat labile at 100° C., but it is not completely destroyed by refluxing the urine sample for one hour. The urine sample may be concentrated by vacuum distillation at 38° to 40° C., and the depressor substance is recovered almost quantitatively in the residue from the distillation.

The apparent activity of the depressor substance is increased by bilateral vagal section; it is decreased by subsequent bilateral carotid artery ligation. Intravenous injection of 200 mg. of tetraethyl ammonium chloride (Etamon) and injection of 10 mg. of benzazoline gydrochloride, 2 benzylimidazoline hydrochloride (Priscol) increase the depressor response to the urine. The possible relationship of this substance to hypertension is discussed.

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EFFECTS OF COLD ON INFANT RATS: BODY TEMPERA-
TURES, OXYGEN CONSUMPTION, ELECTRO-
CARDIOGRAMS[1]

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THERMOREGULATION in mature animals has been studied especially by physiologists who realized the important contribution of this regulation to the constant internal environment. The stable body temperature puts mammals in a different class from cold-blooded animals, as far as survival is concerned, for they are often able to carry on the necessary functions of finding food and protecting themselves against enemies, almost regardless of the environmental temperature.

The responses of adult animals to cold environments have been well established by Barbour (1), Giaja (2) and others. The development of these responses in immature animals, however, has not been as thoroughly studied. Hill (3) presented measurements of body temperatures, during cooling of rats at different ages, and found that the greatest gain in the resistance to lowering of the body temperature occurred between 18 and 30 days of age. Antoschkina (4) measured oxygen consumptions during experimental cooling of young rats. Her conclusions were that rats are poikilothermic at birth, not showing evidence of chemical regulation until one to two weeks of age, or physical regulation until three to four weeks of age.

The changes in the body temperature, the heat production and the electrocardiogram, during and after single exposures at different stages of development, have as yet not been thoroughly studied and are the subject of this paper. Thermoregulation is concerned with the gain of heat from the environment, with internal heat production, and with heat loss. In cold environments, heat gain from environment, of course, is not a factor. In this investigation, cooling was observed by body temperature determinations and heat production by oxygen consumption measurements. Since the circulatory system is the recognized distributor of body heat, electrocardiographic data were taken to study the condition of the heart in the maintenance of the circulation. Observation of the skin color was the only practical indicator of peripheral circulation.

PROCEDURE

Forty-one white rats from 8 litters of the Wistar strain, of o to 17 days of age, were used. Each rat was exposed, in a sealed modified Fenn respirometer, to a control water bath of 35°C., so its

normal body temperature, electrocardiogram, metabolism and general neuromuscular behavior could be observed. Then the respirometer was removed to the experimental cold water bath of 20°, 10°, 5° or 2° for two hours, usually, in which period the animal's reactions to the exposure were studied; finally the respirometer containing the animal was transferred back to the control 35° bath for rewarming and kept there until the animal was at its pre-exposure temperature. The 35° point was chosen for the control bath because this, coupled with the animal's metabolism, brought the body temperature up to about 37°.

The respirometer was a differential volumeter which permitted an accurate reading of the animal's oxygen consumption at all times except during the severest changes in the temperature of the chamber. The rat lay in the glass vessel of 157 ml. volume on a metal screen above soda lime in an atmosphere of oxygen. Records of the oxygen consumption were kept continuously, except for the first few minutes of each immersion and the data presented in this paper are all corrected by a small respirometer factor and to 0°C. and 760 mm. pressure.

The intraperitoneal temperature was recorded every six minutes by a Brown recording potentiometer, fine-gauge thermocouples being threaded through the abdominal wall from one side to the other. This was found experimentally to be a more accurate measure of the core temperature of the body than was the rectal temperature, since it changed more slowly than the rectal temperature during cooling and warming. Some experiments included records of rectal and surface temperatures, as well as intraperitoneal and air temperatures inside the respirometer.

Electrocardiograms were taken at intervals by means of subdermal steel electrodes leading to a Sanborn electrocardiograph. Lead II was their approximate position.

RESULTS

Intraperitoneal Temperature. The intraperitoneal temperature from birth up to 17.5 days of age depends almost entirely on the environmental temperature. When the animal is in the 35° control bath, the newborn's intraperitoneal temperature is not above that of the bath. However, at 3 or 4 days of age, it is about 1° over the bath temperature and this gradient increases slowly with age, the oldest animal showing a body temperature 3° over that of the bath after about 40 minutes.

This gradient effect may be due to several factors. The larger rat has proportionally less surface area over which to lose heat and more tissue to produce heat. Moreover, the 17-day rat has a coat of baby fur, as well as an appreciably thicker skin and body, to increase the insulation against heat loss.

During cooling, however, the intraperitoneal temperature is almost completely dependent on the environmental temperature. When the respirometer containing the animal is exposed to the cold bath, the body temperatures begin dropping immediately; the colder the bath, the more precipitous the fall. The rate of fall slows and almost plateaus as it approaches the air temperature of the respirometer. In general, with experimental temperatures down to 5°C., the difference which the animal can maintain between its temperature at the plateau and the external temperature is nearly as great as that which it could maintain at the control environmental level of 35°. These results agree in general with Gulick's (5) work on young rats. Figure 1 shows the body temperature of an 8.5-day rat before, during and after a 20° exposure.

A series of similar cooling experiments was done on 5 formalin-injected animals to compare the rate of temperature change of these non-metabolizing animals with the live ones. The gradients between the internal and the environmental temperatures for the dead rats during cooling are almost identical for all ages and weights, less than 1.5°C. They exhibit typical Newtonian cooling curves, which the living rats do not do. The live animals, on the other hand, show a progressive increase in

their ability to maintain a wider gradient for a longer time as they grow older. Since, as will be shown later, the metabolism falls during the cooling, the ability to maintain this gradient may be due, to a considerable degree, to an increasing ability to regulate peripheral blood flow.

However, there is no evidence of this ability to keep the body warmer than the environment during an exposure of 2°C., in my apparatus, even in a rat of 11.5 days and 21 gm.; the gradients of the live and dead animals show the same curves with time. It appears therefore that, if the exposure is severe, the animal shows no ability
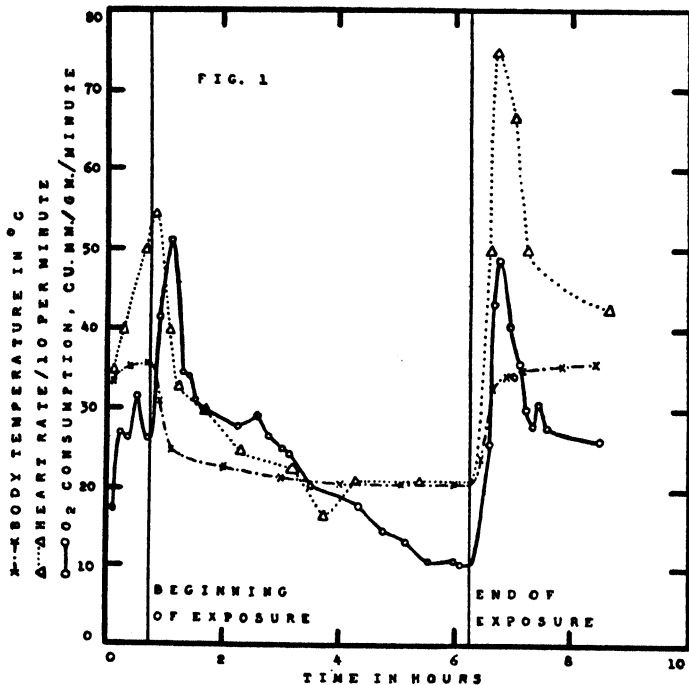
Fig. 1. A TYPICAL COOLING EXPERIMENT, done on an 8.5-day-old, 14.4-gm. rat at 20°C. The responses of the body temperature, heart rate and oxygen consumption are shown during the 35° control period, the 20° exposure and the final 35° recovery period.

to preserve its body temperature. This is probably due to the paralyzing action of cold on the nervous system, preventing the protective reflexes.

There is no correlation between the rate of fall of body temperature and death, nor between the depth of body temperature and death, except that the 4 animals which died, out of the 41 experimented on, died between 3.5° and 7.5°C. Most, however, survived temperatures as low as 2.5°. Apparently there is much variation in individual susceptibility to cold.

The rise of body temperature during the rewarming in the 35° bath was faster than the fall had been, judged by the time required for half completion, because heat production is working toward a rise in temperature during the rewarming and against the fall during cooling. Even animals which eventually died during the rewarming,

and which showed even a faulty electrocardiogram, had enough heat production to cause their rewarming temperature curve to be faster than the cooling curve had been.

   Oxygen Consumption.   From figures 2 and 3, and other data, the following comparisons can be made.   The oxygen consumption/gm. of rat, measured after the animal had become equilibrated in the 35° bath for about an hour during the initial control period, was quite constant with age.   It ranged from 21 to 36 cu. mm/gm. body/min.   When the animals were transferred from the 35° control bath to the 20° bath, those over three days of age immediately increased their O₂ consumption, some-
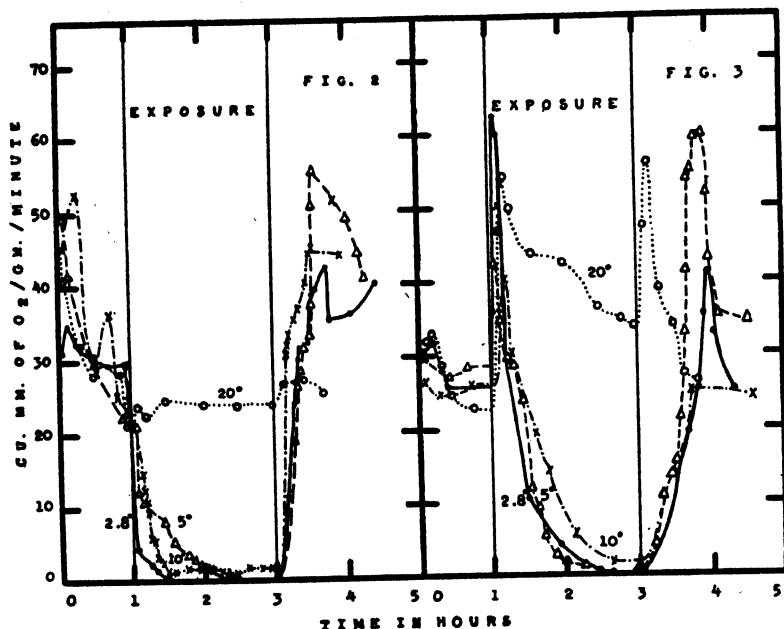
Fig. 2.   OXYGEN CONSUMPTION before, during and after 4 different exposures of 2 to 3-day-old, 6 to 9-gm. rats.
   Fig. 3.   OXYGEN CONSUMPTION before, during and after 4 different exposures of 10 to 11-day-old, 18 to 20-gm. rats.

times to a large degree; the older animals increased theirs by a maximum of 170 per cent (in a 15-day-old animal), within 15 minutes of the beginning of the exposure. This peak metabolism was, however, not maintained for more than one-half hour and then gradually fell to the control 35° level over a one to four-hour period in the 20° bath.   This bath proved to be the most effective one of those tested in stimulating metabolism.

   Animals under about three days of age showed no increase in oxygen consumption at 20°, but just a gradual fall, and even the older animals showed a fall after their peak metabolisms—to a minimum of 35 per cent of the control level in exposures over five hours long.

   On exposure to the 10° bath, only animals over eight days of age showed an increase in oxygen consumption and the highest metabolism recorded for them was 100

per cent over normal, in an 11-day rat. At the lower temperatures, the results were less consistent, but there was a definite decrease in the number of animals which manifested self-protection by increasing their metabolisms. The degree to which they were able to raise these metabolisms likewise fell markedly.

In explaining the apparent decrease in stimulation of metabolism with an increase in the severity of the exposure, probably a large factor is that the colder baths reduce the body temperature too quickly for the animal to mobilize its responses. It was pointed out above that the colder the bath, the faster the fall in body temperature. Cold is well-known as an anesthetic and narcotic and this depressant effect prevents any excitatory response from occurring. The mechanisms involved in increasing the metabolism are discussed later.

In all of the animals experimented on below 20°, immediately after the beginning of the cold exposure or if there was an increase in metabolism immediately after the peak metabolism, there was a precipitous drop in oxygen consumption which slowed as it approached zero. In the 20° exposure, however, animals older than nine days showed the stimulated metabolism only during the first two hours of the exposure, but even this was beginning to fall toward the normal 35° level. Apparently, between the 20° and 10° exposures, there is a break somewhere in the animal's ability to keep its metabolism high. Possibly this break would come at the lethal point for adult animals, between 13° and 16°C. It seems that there is some factor in the adult rat which causes death in this temperature range and it seems possible that something similar, but non-lethal, is breaking down in the young rats also.

It was constantly found that the metabolism fell to zero, as far as could be determined with this respirometer, in all animals up to 10 days of age on exposure to 3° or lower. The older animals were apparently able to maintain their metabolisms at about 0.5 cu. mm. O_2/gm. rat/min. until the end of the exposure. These amazingly low or non-existing metabolic rates and the apparent cessation of visible respiratory movements by no means predicted death. This lack of breathing lasted up to 108 minutes, followed by complete recovery of the animal.

At the end of the exposure, between 20 and 25 minutes after the beginning of rewarming from 5° or lower temperatures and between 15 and 20 minutes after the beginning of rewarming from 20°, the oxygen consumption began to rise at about the same rate as it had dropped and was back at its pre-exposure level within 42 minutes of the beginning of rewarming, the slower ascents being in the colder rats.

The first breathing movements, however, are evident slightly sooner. After 5° or colder, the first gasping movements were seen at 15 to 25 minutes in the 35° bath; after 10°, at 5 or 6 minutes in the 35° bath.

Often the metabolism continued rising, to as much as 100 per cent (an av. of 60%) above the pre-exposure level, and slowly descended to normal within two hours of the beginning of rewarming. The largest increase over normal occurred in those animals exposed to the lowest temperatures, 5° or lower, or to the slightest exposure, 20°. It is difficult to find an explanation for this increase since there was no obvious correlation of appearance of this 'overshoot' with age, length of exposure, depth of oxygen consumption fall during cooling, increased metabolism at the beginning of cooling or with shivering movements, although muscle tension may have been in-

volved.   Nor did there seem to be any correlation with the length of time or degree that the animal was anoxic during the cooling or rewarming, so the increase does not seem to be an oxygen debt.

It is difficult to state with certainty the method by which the metabolism is increased in the infant rats.   As far as muscular activity is concerned, no increase in movements could be detected during the increased metabolism in the rewarming period and, although there was usually some struggling at the beginning of cooling when there was an increase in metabolism, the increase and the struggling sometimes appeared independently.   No shivering movements were observed in the infant rats, even in the older ones.   Muscular rigidity and tonus, however, may be a factor in the augmentation of oxygen consumption of these animals.   Barbour, McKay, and Griffith (1), Dill and Forbes (6) and Swift (7) have all reported that muscular rigidity and tonus are capable, in man and rats, of producing an increased metabolic rate—up to 36 per cent, Swift (7) states—without actual shivering.

As far as hormonal stimulation is concerned, it is interesting to speculate as to the possibilities of increased activity of the adrenal and thyroid glands.   It has been demonstrated by Tyslowitz and Astwood (8) and Giaja and Chahovitch (9) that the adrenal cortical hormones are necessary for resistance against cold.   The difficulty in applying this fact to my experiments lies in the questions of whether the adrenals are actually functioning at birth in rats and whether they or the thyroid respond to stimulation in the short periods involved in these experiments.

Electrocardiogram and Pulse Rate.   The electrocardiograms taken during both control and cooling periods show that the heart rate is lowered almost linearly with a decrease in body temperature (fig. 4).   Crismon (10) and Hamilton, Dresbach and Hamilton (11) likewise found a linear relationship between body temperature and heart rate of adult rats.   The line for the newborn rats, however, is located toward the lower body temperatures.   This is perhaps to be expected since the newborn animal's heartbeats stop at a lower temperature than do the adult animals'.   Adult rats' hearts stopped beating at between 16° and 12.5°C. rectal temperature (11), while the newborns' stopped between 9° and 3.5°C.   Moreover, this asystole was lethal for the adult animals, while it was not for the newborns.

These results suggest that the newborn, incompletely developed rat resembles a cold-blooded animal in its ability to survive asystole during adverse external conditions with no apparent permanent damage.   One rat which we studied lowered its normal heart rate of 860/min. to 12/min. during hibernation.

The rate of conduction of the cardiac impulse slows during cooling in proportion to the slowing heart rate.   This is to be expected, since the cardiac cycle length is inversely proportional to the cardiac rate, and the P-R interval and QRS duration increase linearly with cycle length.   Crismon (10) and Hamilton *et al.* (11) found this to be true also of adult rats and Clark (12) found it in rabbit and frog isolated hearts.

During the cooling experiment there is some increase in voltage of R-waves, as Crismon (10) reports for adult rats, immediately after the beginning of cooling.   This is followed by an enormous decrease in voltage during the decrease in heart rate and conduction rate.

There is apparently no temporary increase in heart rate, as there is in oxygen consumption, immediately after starting the exposure to cold. This is illustrated by figure 4, in which the heart rate of a 9.5-day-old rat is plotted against its body temperature during the control, cooling and rewarming periods of a typical experiment. The heart rate shows a precipitous drop which slows as it approaches a steady rate which, in many cases, as in this animal, was zero.

Occasional records of R-waves at irregular intervals, as the animal becomes very cold, indicate auricular fibrillation although, in general, no irregularity of beat occurs before the cessation of beat. The lack of ventricular systole lasts until just after the beginning of warming, which has meant asystole for as long as 82 minutes with
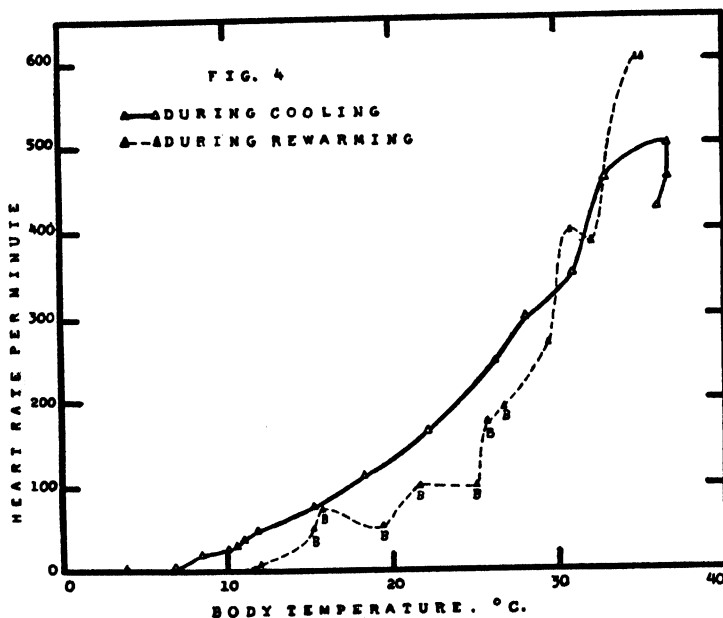
Fig. 4. HEART RATE AT DIFFERENT BODY TEMPERATURES, as a 9.5-day-old rat cools to 2°C and then is rewarmed. The points marked 'B' on the rewarming curve indicate abnormally slow conduction, or 2:1 or 3:1 blocks.

complete recovery of the animal. The only longer records of asystole occurred in animals which recovered their heart beats, but then died during the rewarming. When the animal is rewarmed, the heart beat reappears or increases within a few minutes and reaches the precooling level or higher.

In general, in the 20° experiments, the heart rate is slightly higher during the rising (recovering) body temperature than during the same body temperature as the animal cools. In the 2° experiments, however, just the reverse is true; the heart rate during rewarming is slower than during cooling, at the same body temperature (fig. 4). The explanation for this difference may be that in the 2° experiments, the electrocardiograms show conduction disturbances during the first part of the rewarming period, and these may account for the slower heart rates. It is also possible that

the heart may be warmer or cooler than the abdominal temperature which was the one measured.

Moreover, the heart is often faster in the final control period than during the initial control period; where the data were continued long enough, they indicate that this is followed by a slow fall to the normal level. This 'overshoot' is exemplified by figure 4. Possible explanations for the overshoot might be that heating is a more effective influence upon the cardiac apparatus than is cooling. Another explanation might be that the slightly anoxic cooled animal responds to cardiac-controlling reflexes more than does a warm, well-oxygenated animal. There is also the possibility that accumulated metabolites might stimulate the circulation during and after the warming.

The electrocardiographic data suggest a prognostic sign for the non-survival of the animal. Many of the rats developed abnormally slow conduction of the cardiac impulse, or even 2:1 or 3:1 blocks during the rewarming, but if the animal developed irregular QRS impulses, along with the block, apparently more serious injury to the auriculo-ventricular node and ventricular conduction system had occurred and the animal did not survive. Only irregularly can a sinus impulse reach the ventricular conduction system and musculature. Moreover, the ventricular pacemaker, the A-V node, is unable, apparently, to initiate its own rhythm effectively. All of the ventricular impulses then come from the sinus and the injury is apparently progressive. Fewer and fewer ventricular systoles occur and the animal dies within half an hour.

Occasionally, during rewarming of the animals which eventually survive, very slow conduction is followed by a 2:1 or 3:1 block as the parts of the heart become more normal physiologically. This seemingly paradoxical situation is due to the fact that, during the former condition, the sinus frequency is so slow that the ventricle is non-refractory by the time the slowly travelling impulse gets to it. This changes to a 2:1 or 3:1 block as the sinus frequency becomes faster, because conduction in the A-V node and bundle have not correspondingly improved, so the ventricle is still refractory after every second or third sinus impulse. This condition, however, soon disappears, leaving a physiologically normal heart as ventricular conduction improves. Occasionally, in some animals during rewarming, the ventricles developed a rhythm different from that of the auricles, but none of these animals survived this type of block.

It seems probable that the blocks produced in the rewarming of the infant rats were results of anoxia. Lewis's (13) description of the electrocardiograms of mature rats during progressive anoxia is identical with these results. His blocks readily disappeared when ventilation was restored if the asphyxia were not carried too far, and he believed that the obstruction was in the A-V node, because more peripheral parts of the conduction system could still conduct. When it is considered that tissues with a high metabolism are especially sensitive to anoxia, it seems reasonable that the presumably high glycogen-containing, high metabolizing tissues of the cardiac conduction system should be the first and perhaps only ones to be injured by anoxia.

Probably the cooling of the newborn rats produced too rapid a lowering of the metabolism of the whole body to cause anoxia. However, while the heart stops

beating, and oxygenated blood is therefore not being supplied to the body for over an hour, some body metabolism still exists and, as a result, the body and blood are becoming very slowly anoxic. When the animal is rather suddenly warmed, the metabolism suddenly increases and the heart and respiration may start too slowly at first to supply the sudden large needs for oxygen. The anoxia more severely interferes with the function of the cardiac conduction system than other tissues, so temporary heart blocks appear until the anoxia disappears and, if the anoxia has been severe enough, the injury is irreversible and the animal dies.

Effects of anoxia on dogs' hearts include congestion of the heart and veins with dilated auricles and pale contracted ventricles (14). The autopsies of the newborn cooled rats also showed these signs, all of which appear to be indications of the inability of the anoxic heart muscle to do its work. Alexander's (15) report of the German experiments on man mentions the finding of right heart and pulmonary engorgement in humans. Crismon (10) found the same in cooled adult rats and that the A-V blocks in his cooled adult rats disappeared during artificial ventilation. This may indicate that a deficient respiratory exchange adds to the deficient circulation. It is also possible that cold per se might cause enough injury, in some way, to produce these abnormalities. There were no detected cardiac abnormalities still existing at the end of the rewarming period when the surviving animal was removed from the respirometer.

In plotting heart rate, at 35°C., against age, it was noted that the heart rate is lower in the younger animals than it is in the older infants. Marcuse and Moore (16), who did not keep the body temperature constant, found the same thing in newborn rats. They point out that kittens and pigs have faster heart rates when very young and the rates decrease to the adult values. This phenomenon apparently depends on the species.

Survival. Of the 41 live rats tested, only 4 did not survive the experiment. Two died as a result of 5° exposures and 2 of lower temperatures. Permanent cessation of heart beat occurred in 3 during the rewarming period rather than during the actual cooling and in one, during a 3° exposure which was of much longer duration than any other, nine hours. There is no apparent correlation between non-survival and degree of exposure, except that all died at body temperatures of 7.5° or under. Many more animals, however, survived body temperatures as low as 2.5°C. There is also no correlation between death and rate of fall of body temperature, rise of body temperature on rewarming or age of the animal.

In exposures to 5° or lower, the majority of the animals showed cessation of heart beat followed by cessation of oxygen consumption, often for over an hour. Neither heart beat nor breathing, however, were required for survival of the animals. Most of the animals began to breathe and exhibit heart beats within a few minutes after the beginning of rewarming, the appearance of the heart beat again preceding the metabolic change. It is probable that these animals were not anoxic during the cooling period. It seems more likely that it is not until the rewarming period, when they are warmed enough to increase their metabolisms, that they experience any anoxia. The cardiac conduction system may be sensitive to anoxia in the newborn, though less than in the adult, and it may be irreparably damaged. This would

cause deficient circulation which would increase anoxia in the whole animal, as it is warmed, to an extent which would cause death.

The temperatures from which the newborn rats do not recover (7° or below) differ markedly from those of adult rats (14°–16°). This difference may be attributed to several factors. The metabolism of the younger animal, per unit weight, is smaller, according to Himwich *et al.* (18); Negelein (17) reports that it also has potentially greater anaerobic ability. Therefore, anoxia would not as easily injure the brain of the newborn, which Himwich (18) believes is the limiting organ in tolerance to anoxia. That anaerobic metabolism is a method by which the young are able to withstand anoxia is indicated by the experiments of Enzmann and Pincus (19), which show that the rate of glycolysis in infant mice decreases with age, and Himwich's (18) finding that lactic acid production is higher in newborn rats, and iodoacetate and fluoride shorten the survival period in nitrogen; they conclude that anaerobic metabolism of carbohydrate is an important factor in the survival of anoxia in animals of different phases of development.

Fazekas, Alexander and Himwich (20) point out that newborn rats are less mature in development at birth than are dogs and guinea pigs and correlate this fact with the finding that newborn rats are able to survive anoxia longer than they. This tolerance, they point out, is lost with age. The animals become progressively more sensitive to oxygen lack, presumably because of the development of phylogenetically newer, more easily damaged parts of the brain. Newborn rats over periods of an hour or two seem comparable with cold-blooded animals in their ability to withstand extremely low body temperatures.

SUMMARY

Newborn white rats of 0 to 17 days of age were cooled to 2°, 5°, 10° or 20°C. in a respirometer filled with oxygen and immersed in a water bath. The immediate fall in the intraperitoneal temperature of the rats indicated that the rats were too immature to maintain body temperatures above environmental temperatures.
all temperatures below and including 10°C., the exposures were too severe to elic any but a transient increase in metabolism (as shown by oxygen consumption) and these transient and comparatively feeble protections against cold were manifested only in rats over three days of age. Ultimately, in all experiments on rats up to 10 days of age at 3° or lower, the metabolic rate reached zero and remained so for as long as 108 minutes, followed by complete recovery of the animal.

Electrocardiograms showed that the rate of the heart beat and of the conduction of the cardiac impulse decreased linearly with the decrease in body temperature, the heart beat disappearing when the body temperature reached between 9 and 3.5°C. The asystole lasted up to 82 minutes, followed by complete recovery of the animal and its acceptance by the mother rat. It was believed that the prognosis for non-survival of the 4 rats, which died out of the 41 exposed, was the finding of 2:1 or 3:1 block with irregular QRS impulses, suggesting serious injury to the A-V node and conduction system which apparently was irreversible. This lethal effect was manifested only in animals whose body temperatures were 7° or under and the anoxia during the rewarming period may have been the ultimate factor in survival or non-survival. These low lethal temperatures classify the newborn rat as a temporary poikilotherm.

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TOLERANCE TO COLD AND ANOXIA IN INFANT RATS[1]

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T HE object of this investigation was to trace changes with age in the ability of infant animals to survive hypothermia. It has been known since the observations of Edwards (1) were published in 1824 that infant mammals survive the cooling of their bodies to lower temperatures than adults of the same species tolerate. The limits in temperature and in time that can be endured have not been ascertained for any species; the question arose whether indefinite poikilothermic existence was possible at certain ages.

Infants of various species have been known to survive lack of air or oxygen for considerable periods of time, ever since the observations of Robert Boyle (2) recorded in 1675 and the unrecorded experiences of obstetricians and stockbreeders of all centuries. What is the relation of the two tolerances, that to cold and that to anoxia? Do they disappear at the same age? If either or both tolerances could be preserved into adult life, considerable understanding of the animal's limitations toward cold and anoxia would be obtained.

The present investigation started from the observation that infant rats survived a body temperature of 2° to 5°C. for two hours when enclosed in an atmosphere of oxygen (3), but not when the body was immersed to the shoulders in water and the head was surrounded by air.

PROCEDURES

The initial technique consisted in fastening rats to small boards by passing adhesive tape about each leg. Each infant rat had a thermocouple of fine nylon-wrapped wires (iron and constantan) inserted 1 to 3 cm. (depending on body size) into the colon, the wires being taped to the animal's tail. The potential differences, between colonic couple and reference couples to copper at 0°C., were balanced every six minutes with a recording potentiometer. Thermocouples were calibrated at the close of each experiment. After a period in room air the boards were clamped so that each of 5 or 6 rats of known ages were held in water of regulated temperature. Ordinarily the infant rats were immersed to the shoulders and neck; the trunk and limbs were covered, but the head was clear of the water even when the animal struggled. For a given experiment the stirred water was kept at a set temperature by a toluene-mercury regulator and relay connected to a compressor that supplied a refrigeration coil.

In testing the effects of various gaseous atmospheres, infant rats were placed in 250-ml. Erlenmeyer flasks, usually with thermocouples in their colons. The flasks were at first surrounded by water of 35°, later by water of a low temperature and finally by water of 35°. A stream of air or nitrogen or oxygen at room temperature passed continuously through each flask.

Survival was judged not by the maintenance of particular activities, for often all movements and reflexes disappeared, yet the animals revived. Instead, the criterion was that of recovery and continued viability when the rats were rewarmed at 35° with the head surrounded by air. Therefore, individuals (usually in pairs) were removed from cold at diverse times, the aim being to have some survive and some die. Lethal effects of cold and of anoxia turned out to differ enormously in infant rats of diverse ages. Therefore it became necessary to work out systematically, for a series of post-natal ages, the lengths of time for which given body temperatures could be endured.

Rates of body cooling differed greatly, depending on whether the infant rats were in water or in flasks (fig. 1). The course over which the colonic temperature decreased were only roughly similar in the two environments and in two temperatures.
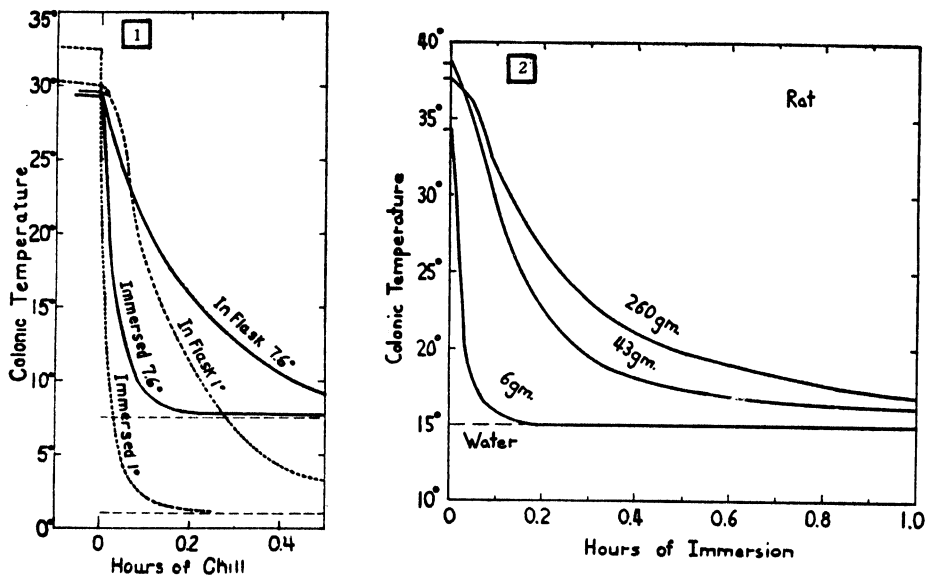
Fig. 1. COOLING CURVES OF INFANT RATS aged 9 days (wt. 15 gm.) under 4 conditions. In water of 2 different temperatures as indicated, rats were either immersed to the shoulders or were placed in a glass flask surrounded by water. Each curve represents the mean of 3 to 6 individuals simultaneously exposed, all of which survived treatment.

Fig. 2. COOLING CURVES FOR colonic temperatures in rats of 3 sizes that were immersed to the shoulders in water of 15°C. at zero time. Each curve represents the mean of 5 individuals simultaneously exposed. Their ages were 1 day, 27 days and about 200 days respectively.

For purposes of estimating the endurance times at diverse temperatures, the arbitrary rule was adopted of calling zero time when the colonic temperature passed 15°C. But in anoxic exposures, the rule was to start passing the nitrogen when the colonic temperature came within 4°C. of the water temperature and to call the initial passage of the gas zero time.

Rates of cooling differed with age and body mass (fig. 2). When the rats were immersed in water the courses of cooling were similar excepting for their time constants. However in air, rats down to 26 days of age could not be cooled more than a few degrees and those down to 10 days of age resisted cooling often for some hours so long as they were breathing. In nitrogen, however, the resistance to cooling disappeared. In general the colonic temperature ultimately came to lie within 2°C. of the water temperature; the younger the individual, the more nearly did the difference approach zero.

Immersion in Water, Air around Head. The endurance times varied enormously at diverse temperatures (fig. 3); a colonic temperature of 2°C. was endured

for only half an hour, 10° was endured for over two hours. Evidently precise knowledge of the temperatures of the vital tissues was the key to recognition of lethal conditions. Endurance times also varied with age, becoming shorter at any given body temperature. Probably the change was gradual as the animals aged, but in this series the data accumulated were only sufficient to show the marked diversity between two age groups (fig. 3).

Adult rats were unable to endure a deep temperature of 14° for even six minutes and usually died in two hours at 15°. Above that temperature long exposures appeared to be tolerated. Infant rats not only endured much lower temperature, but exposure time made a larger difference.

Since rate of cooling differed in animals of diverse sizes (and ages) (fig. 2), it could be supposed that slow cooling might yield other results in infant rats. In one test, the several individuals were placed in water of 13° and the temperature of the water was lowered during an hour's time to 8°. Survival was not extended by the
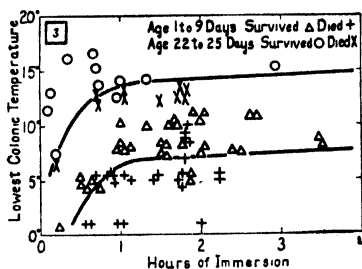
Fig. 3. COLONIC TEMPERATURES of infant rats in relation to the length of time those temperatures remained below 15°C. Each rat was immersed to the shoulders in water and the head was surrounded by *air*. Two age groups are represented; a curve for each group separates survivors from non-survivors.

slow cooling. The slow cooling characteristic of exposures in air was also little different (see next section).

Obviously the tissue temperature was of more lethal consequence than the external temperature. The endurance times in the graphs represent the lowest colonic temperatures attained for the period of time that the colonic temperature remained below 15°C. An inevitable feature of this form of comparison is that the younger the animal, the longer was the exposure to those low temperatures that approached the minimal, for it had cooled faster than older animals.

No evidence was found that rate of rewarming was of consequence. Actually, recovery depended upon artificial rewarming, especially in the youngest rats, for heat production was almost abolished in them (3) and heat could be received from room air only slowly. Infant rats often died if not fully rewarmed before they were returned to the mother; they were rejected by the mother more frequently when they were hypothermic.

Partial immersion allowed survival at colonic temperatures that were intolerable during immersion to the neck. Ordinarily the hind legs, tail and a small portion of the abdomen were then covered by the water. In such immersions the head and chest were measurably warmer than the colon. To ascertain this fact, thermo-

couples were sometimes placed in the upper esophagus. Moreover, the differences between the two regions increased with age; at 25 days the difference was only 1°; in the adults a gradient up to 4° was found.

Initial experiments seemed to indicate that immersion of the chest had a more lethal effect than partial immersion. Subsequent tests showed that insufficient numbers of animals had been used. Any residual difference is now believed to be due to the fact that colonic temperatures did not represent the temperatures of vital regions during partial immersion. No factor other than temperature of chest or head has been demonstrated to influence the lethal conditions for infant rats breathing air.

Rats cooled below 10°C. did not breathe in any visible fashion. It seems likely that no air was pumped into the chest, but over periods greater than 1.5 hours, oxygen was indispensable, as will be shown later. Complete submergence was found to bring death in less than one hour; it was probable that water filled the respiratory passages during such treatment.

Other movements besides breathing were retarded as the infant rat cooled. Below 8° movements were rarely seen at all, even in response to prodding of the skin on the head. Total cessation of movement was no criterion of ultimate survival; this was the reason that the fate of each animal was ascertained by rewarming after a predetermined period of exposure to cold immersion.

Movements often reappeared in one to two minutes of rewarming. Occasionally a rat that had been totally inactive in the cold required 10 to 20 minutes for recovery. Sometimes total inactivity occurred, the cold animal having a blue skin instead of the more usual pink. It is possible that blueness means cessation of heart beats, for electrocardiograms ceased to show cardiac conduction at some such times. In general, heart beats were no criterion of survival, for they might cease after cooling was over and often may resume after long cessation. Resumption of beats was also no prophecy of survival, since beats occasionally failed after returning for some period; this fact was ascertained by opening the chest as well as by recording the electrocardiograms.

Infant rats that died hypothermally revealed little that was unusual at autopsy. The lungs were pink or red instead of the white which characterized those that recovered. Probably more blood was contained in the pulmonary blood vessels during chilling; whether this fact was related to failure of survival in certain individuals is not known.

Infants of a few other species were tested by immersing them to the neck. Dog pups survived two hours with final colonic temperatures of 10°C. Two kittens, four days of age, survived 1.5 hours at colonic temperatures below 15° and down to 7.2° and 9.2°. Five others were killed during 1.8 hours between 15° and 5.8°. In another test, 2 two-day old guinea pigs were killed in only seven minutes of exposure between 15° and 13°C. of colonic temperature. That guinea pig infants are intolerant to cold was noted by Edwards. The same species is distinguished by the intolerance to anoxia in newborns (4). It is possible to say that guinea pigs are born in a state so mature that they have little tolerance either to cold or to anoxia; it remains to be ascertained whether they ever had either.

The above data reveal the marked effect of temperature that modifies the survival times of exposed rats. At 20 to 25 days of age the ability to endure temperatures less than 15° is completely lost. The possible constituents of this infantile ability to endure lower temperatures will be considered later in this report.

Cooling in Air. In this series the infant rats were kept in flasks surrounded by water of the desired temperature. At ages up to nine days they were cooled in the flasks; older ones were cooled initially by dipping them in the cold water itself, after which the low temperature persisted while they stayed in flasks. The data may
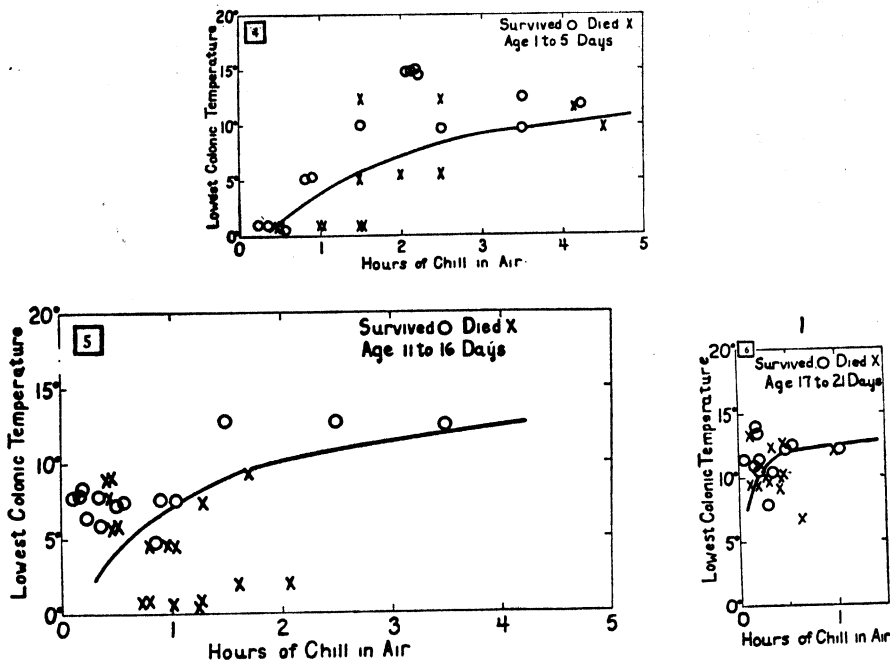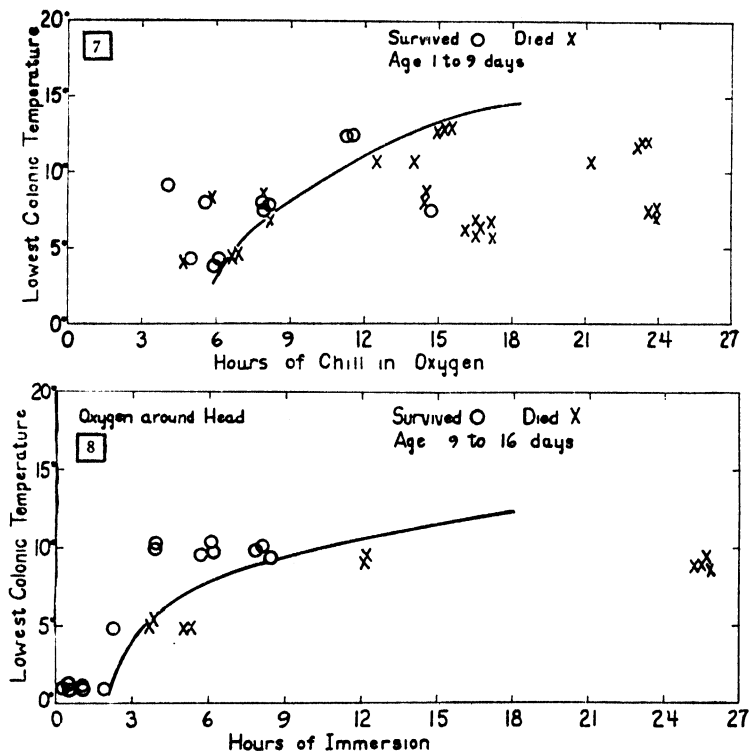

Figs. 4, 5, 6. COLONIC TEMPERATURES of infant rats in relation to the length of time those temperatures remained below 15°C. Each rat was kept in a flask surrounded by cold water; *air* slowly passed through the flask. Three age groups are represented; a curve is drawn in each group to separate survivors from non-survivors.

conveniently be referred to three age groups, though more exhaustive tests might show intermediate tolerance curves at intermediate ages.

The most complete determinations fell in ages one to five days (fig. 4). In this age the curve has nearly the same position as for the infants of similar age immersed in water (fig. 3). It is likely that the two curves would be identical in a larger number of tests.

The tolerance to cold was lost gradually, so that the endurance time in each temperature diminished between the ages of 1 and 20 days (figs. 5, 6). The endurable temperature for some one period of time became higher. The curves are roughly of a hyperbolic character, suggesting that cold and time together tend to yield a lethal product.

Variations of the exposure in air were made as follows: *a*) The infant rat was strapped to a small board as in the immersion tests, then covered by a thin rubber sack and immersed to the neck in water. *b*) It was placed in a flask and enclosed in a refrigerator. *c*) It was placed in a glass cylinder that was immersed in water while a constant stream of air flowed through the cylinder. In each variation only one point on the temperature-duration curve was established. None of the procedures

Fig. 7. COLONIC TEMPERATURES of infant rats in relation to the length of time those temperatures remained below 15°C. Each rat was kept in a flask surrounded by cold water; *oxygen* slowly passed through the flask.

Fig. 8. COLONIC TEMPERATURES in relation to time below 15°C. The infant rats were immersed to the shoulders in cold water and the head was covered by a cowl through which a stream of *oxygen* passed.

modified the results so long as lethality was referred to the tissue temperatures of the animals. Hence rate of cooling and of warming may also be said to be of no consequence.

The fact that infant rats endured lower tissue temperatures than adults was recorded by Wiesner (5) and Antoschkina (6). The time relations were not investigated heretofore and it came to us as a surprise to find that survival was limited in the lowest temperatures to half an hour, while at 10° endurance times up to five hours prevailed. In natural conditions the importance of nesting and the indispensability of periodic rewarming by the mother rat are apparent.

Cooling in Oxygen. The experiments of Fairfield (3) showed that infant rats in oxygen survived at least two hours at body temperatures of 2°C. and at least five hours at 10°. It seemed important to see what endurance limits could be obtained at these temperatures (fig. 7). Evidently cold could be endured for a much longer time in oxygen than in air. This fact indicated that tissues were being injured not merely by remaining at a low temperature for too long, but that a higher pressure of oxygen deferred the injury for some hours. At the same time, no oxygen consumption could be detected at 2° to 5° (3). In temperatures below 6° the heart usually ceased to beat (3); in such case a reserve of oxygen may exist in the animal when the oxygen has been supplied at high pressures during cooling. Oxygen may fill the lungs where it can be picked up by the blood whenever it is circulating; oxygen may be utilized by direct diffusion when the circulation has ceased.

No difference of survival was found between rats of one to five days of age and those of six to nine days of age in oxygen. The former were smaller and naked, while the latter had thicker skins as well as thicker torsos. If diffusion from the body surface were concerned in supplying oxygen, a marked difference would be expected.

Immersion in water, oxygen around head. At ages beyond nine days, infant rats were cooled more conveniently by immersion to the shoulders. When an atmosphere of oxygen was kept around the head (fig. 8) survival was much longer than in an atmosphere of air.

This result confirmed that of the previous series. It also showed that the aid of oxygen in prolonging survival extended to at least 16 days of age. Three tests made at 27 days of age at 14° showed that oxygen no longer protected rats from cold as compared with litter mates that had air around the head. The result also showed that the oxygen had its chief effect in the head or lung region and not over the general body surface. An effect was exerted whether the chest was breathing or not and whether the heart was beating or not.

Cooling in Nitrogen. The remarkable ability of the newborn mammal to survive without breathing or oxygen has been recognized for at least 300 years. William Harvey (7) in 1651 stated that, "I have often seen the human fetus extracted alive from the uterus when the mother has been dead some hours. I have also known the rabbit and hare to survive when extracted from the uterus of the dead mother". Similar observations upon dogs were made by Boyle; he also demonstrated that newborn cats would survive in rarefied air much longer than adults, though breathing and heart beat were suspended for some time. The history of the subject was recorded by Legallois (8) in 1824 who made important contributions of his own.

That temperature might affect the anoxic survival was found by Edwards (1) in 1824 who submersed newborn kittens in water of various temperatures. Movements persisted longest (up to 49 minutes) in water of 20°C.; their duration diminished sharply in water of both higher and lower temperatures (30°, 42°; 10°, 0°).

It is well known that the tolerance to anoxia diminishes gradually with age, as was shown in various ways for rats by Bert (9), Reiss (10), Cameron (11), Fazekas *et al.* (12), Selle (13), Hiestand (14) and Cheymol (15). For the most part, however, these investigators neglected to control the animals' temperatures. Could it be that the tolerance to cold depended upon the tolerance to anoxia and was lost simultaneously with it?

Rats were therefore exposed to an atmosphere of nitrogen under specific conditions of body temperature.   The infant rats were first cooled, then the air of the flasks which contained them was replaced by a rapid and steady stream of cylinder nitrogen.   The length of time that infant rats could survive in absence of oxygen was, under the most favorable conditions, two hours (fig. 9).   Tolerance times were greatest at about 10°.   They diminished markedly and sharply at temperatures below 5°.
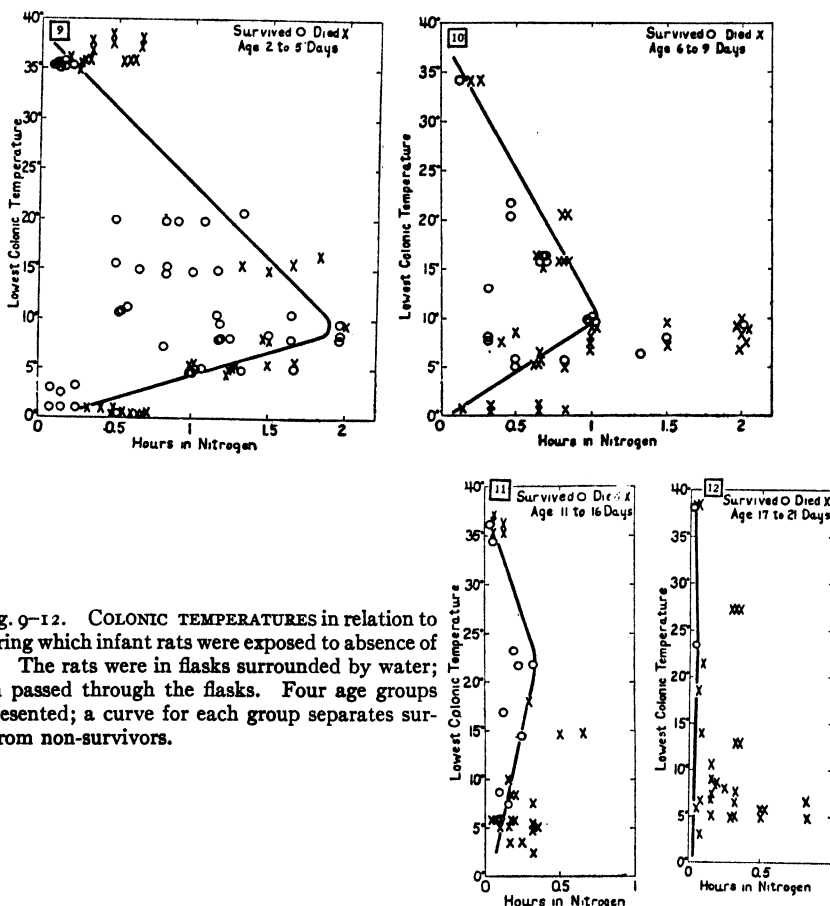

Fig. 9–12.   COLONIC TEMPERATURES in relation to time during which infant rats were exposed to absence of oxygen.   The rats were in flasks surrounded by water; nitrogen passed through the flasks.   Four age groups are represented; a curve for each group separates survivors from non-survivors.

Tolerance times also diminished with age (fig. 10); so that by 11 days of age (fig. 11) the maximal time was only 0.3 hour and by 17 days (fig. 12) was that of the adult. The optimal temperature for survival in nitrogen increased from 9° in the newborn to somewhere between 15° and 25° at 12 days of age.   In contrast to the tolerance in nitrogen, that in air was much greater at temperatures above 5° and lasted later in life, e.g. 11 to 13 days (fig. 13).   In other words, tolerance to anoxia was largely lost before tolerance to cold was lost.   The two appear to be independent in large part.

Oxygen aided survival even in temperatures below 5°, where air was scarcely of more aid than nitrogen and was effective over air at an age (16 days) at which anoxic survival had disappeared.

Further information about the effects of oxygen was gained from the following tests: *a*) Oxygen was injected into the peritoneal cavities of 7 infants of three days of age before they were cooled to 5°C. No appreciable difference occurred in survival when compared with an equal number of uninjected litter mates. *b*) Rubber collars were placed around the neck and sealed to it by rubber cement. Nitrogen was then passed to the head in 13 animals and to the trunk in 10 animals, while air irrigated the other compartment. In general at 8° those that had air around the head survived indefinitely longer than those with nitrogen around the head, even though no breathing could be detected. *c*) In 6 more infants an atmosphere of oxygen was kept around the trunk while nitrogen surrounded the head; they also survived in no greater proportion than when nitrogen bathed the whole body. It is concluded that oxygen is of no aid in survival except when it surrounds the head,
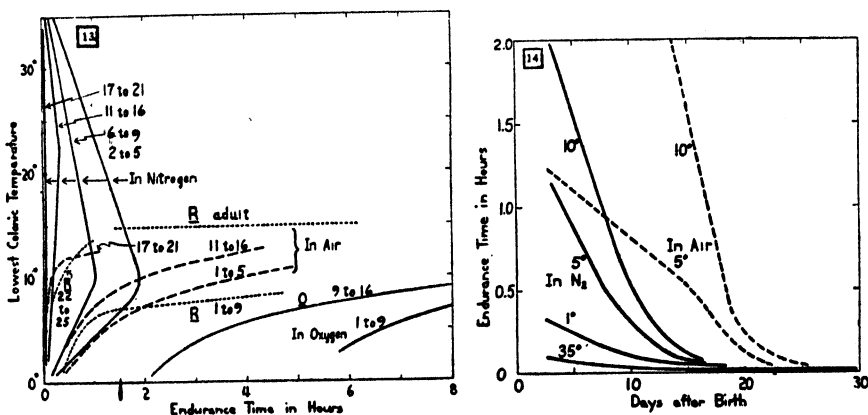
Fig. 13. COLONIC TEMPERATURES in relation to mean endurance or survival time in rats o various ages. The 12 curves are traced from preceding figures (adult is from next paper) and are fitted to common coordinates. Numbers refer to days of age; *R* refers to rats immersed in cold water to the shoulders, head surrounded by air; *O* refers to similar immersion, head surrounded by oxygen.

Fig. 14. ENDURANCE OR SURVIVAL TIME in nitrogen and in air in relation to age of infant rats. Each curve represents the body temperature indicated, as read off from figures 4–6 and 9–12.

whether breathing is going on or not. *d*) Glucose in high concentrations in tissues might prolong anoxic survival. In 14 tests glucose was injected intraperitoneally before 2- to 4-day-old rats were cooled to 5° or 8° and placed in an atmosphere of nitrogen. The same proportion survived as among uninjected rats. This result at low body temperatures does not agree with the increase in anoxic survival reported by Himwich *et al.* (16) and Selle (17) in infant rats that were presumably much warmer.

In sum, the survival of infant rats in the absence of oxygen proved to be highly sensitive to body temperature. Most of the anoxic survival disappeared in the first 10 days of life, while the survival in cold air lasted a second 10 days longer. Surrounding the body by air or oxygen while the head was in nitrogen did not aid nor did the injection of glucose.

Comparisons of Three Variables. The effects of various combinations of three

influences upon survival of infant rats have now been reported; they are age, body temperature and time. By transforming the coordinates, several additional features of the combined influences can be pictured. The interpolated results are available in figure 13.

When body temperatures were kept constant, the tolerance times diminished with age along the curves shown in figure 14. The curves have rather similar shapes whether the tolerance to low temperatures was tested in nitrogen or in air, but the time scales are very different. The ages at which tolerance was lost in the two atmospheres differ greatly. These curves represent the changes in those processes that limit the rat's tolerance. They are curves of growth in intolerance. The curves of figure 14 correct to constant temperature the similar curves that have been
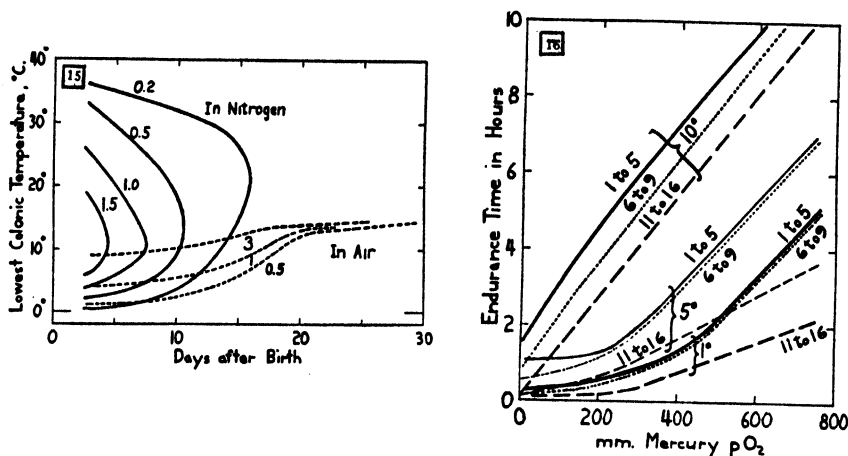
Fig. 15. RATS WITH VARIOUS BODY TEMPERATURES survive for the diverse periods of time indicated, when they are of the ages shown upon the abscissae. Curves are derived by transforming interpolations in previous figures.

Fig. 16. ENDURANCE TIMES of infant rats in relation to the oxygen pressure to which they are exposed at the several body temperatures indicated. Infants of 2 ages are represented. Curves are derived from previous figures.

constructed by those investigators who measured the tolerance times to anoxia at various ages without controlling the body temperature that prevailed. In their results the younger animals undoubtedly had lower temperatures than the older ones.

Figure 15 presents the contours for equal survival times at diverse ages and body temperatures. These contours define the safe conditions both in nitrogen (absence of oxygen) and in air. While during the first week of life, temperatures below 5° could be endured for about the same length of time regardless of the presence or absence of air, by the age of two weeks the tolerance to anoxia had been lost at all temperatures, leaving the tolerance to cold little impaired. Differentiation of the two tolerances is again evident.

Finally, interpolations at diverse pressures of oxygen are shown in figure 16. In 10° the hours of life added by each increment of oxygen pressure were about the same as in 1°, but advancing age eliminated the survival at low pressures before it

diminished greatly the time before death at high pressures.   The results also suggest that survival may be greatly prolonged at oxygen pressures above one atmosphere.

COMMENT

Outstanding among the results of this investigation is the discovery that oxygen aids prolonged survival at body temperatures below 12°C. Breathing then has usually ceased. Thereafter it may be the supply of oxygen available in the lungs which is important. If so, the circulation of blood might transport the oxygen to other tissues. But below 6° there is no heart beat. Hence oxygen is reaching some critical tissues by diffusion through other tissues. The reserve of oxygen was shown to be either in the lungs or around the head. The fact that the reserve does not last indefinitely suggests the former. It might be inferred that in larger species of the same postnatal age, diffusion distances would be too great to allow such a large effect of oxygen. Whether this is so has not been ascertained.

Anaerobic survival of the young infant rat occurred at all temperatures. It was longest at 10°. Above that temperature it may be inferred that metabolism is slowest at the lower temperatures, so that a reserve lasts longer. Below that temperature some limitation prevails that has a negative temperature coefficient. It seems likely that this activity is the same temperature-sensitive one that also limits aerobic survival. In part this limitation is also oxygen-sensitive. It is clear, then, that cold has a lethal effect below 10° that is not limited by metabolic reserves, but perhaps represents the destruction of enzymic and other systems by low temperatures. The inference is drawn that cold is destructive in infant rats, at about the same temperature (8° to 10°) that it is destructive to peripheral tissues in adult rats and rabbits. The heart may be a limiting tissue in aerobic survival, but there is no evidence that such is usually the case in infant rats. The heart's activity is here divorced from survival. The heart very often resumes beating during rewarming, though this event alone does not foretell survival for other irreversible damage may have been suffered.

The minimal temperature for heart beats rises gradually with age, from 6° in the newborn to 15° in the 27-day-old rat. . The parallelism of this rise with the rise in minimal aerobic survival suggests that they are interrelated.

Breathing movements serve as signs of life at temperatures above 15°. Survival is certain as long as gasping (jaw movements) continues. Observers (13) distinguish an aerobic series of gasps from a subsequent, less frequent, anoxic series. After the last gasp, survival is no longer possible, but at temperatures below 15°, breathing movements are usually absent. No longer do they serve as signs of survival; they are suspended in cold and are readily resumed during rewarming. Occasionally artificial breathing may be imposed with probable advantage, but in most tests here it has been omitted. In certain instances foamy fluid issued from the mouth when the chest was squeezed and thereafter gasping was resumed. Hence it is always possible that mechanical obstruction is sufficient to influence spontaneous activities that would otherwise secure survival. Other movements such as pendular limb movements, and responses to mechanical irritation, occasionally serve as signs of life but cannot be counted on. They may well depend upon portions of the neuromuscular system that do not determine ultimate survival.

At times skin color was thought to indicate viability. The majority of chilled infant rats had bright pink surfaces, but some were blue or dusky. The pink ones usually recovered as soon as rewarmed, but occasionally not at all. The blue ones rarely recovered promptly, but in some instances resumed gasping, then breathing, then other movements, after many minutes of rewarming. There seems, therefore, to be no combination of signs by which the capacity to recover from chilling can be foretold.

SUMMARY

Infant rats of ages up to 27 days were cooled to known body temperatures, either without or with immersion in water. After various times of exposure they were rewarmed to ascertain survival; all signs of life may be absent during hypothermia. In an atmosphere of nitrogen, newborn rats survived for two hours at 10°C., for shorter times at lower or higher temperatures. The presence of air enhanced survival above 5° and an atmosphere of oxygen prolonged it greatly at all temperatures up to 12°. With increasing age the tolerance to anoxia was lost before the tolerance to cold. This is evidence that these two remarkable tolerances do not depend upon the same critical process.

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LETHAL LIMITS OF COLD IMMERSION IN ADULT RATS[1]

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THE object of this investigation was to ascertain what conditions limit an animal's endurance of immersion in cold water. Is the limit set by the temperature to which the body or certain tissues of it are chilled? Can the limit be changed by previous treatments? How variable are the endurances and susceptibilities among individuals?

Methods of testing tolerances to cold are numerous and diverse. Local or general cooling can be employed; any of a score of responses can be observed. Each of the responses represents a particular combination of functions and properties of the organism. No one test of resistance to cold will assay all the factors that may play a part in tolerance. The survival test here used, however, constitutes a practical over-all test.

Studies of cold *immersion* have been performed by other investigators; few (1, 2, 3) have employed small mammals. One of the questions to be answered here is whether or not cooling by immersion is intrinsically different from any other cooling. Only limited studies of hypothermia have in the past been made without anesthesia; it is important to know whether the anesthesias used by others influenced the extremes of temperatures that could be endured.

PROCEDURES

In the present work, albino rats were restrained by taping their legs to wooden holders. Thereafter they were immersed, usually to the shoulders, in water that was (in most experiments) of a temperature that could be endured by some individuals for two hours. Thereafter the rats were withdrawn from the water and allowed to warm in room air. Only those that survived for an indefinite period thereafter were considered to have endured the hypothermic experience. These conditions of cooling were chosen because they paralleled those under which men are placed during immersion in open oceans.

To measure the temperatures attained within the body, thermojunctions were inserted 5 cm. within the colon. The wires leading from the junction were held in place by taping them to the tail. Sometimes additional junctions were placed in the esophagus; in this case the wires were encased in metal tubes that prevented the rats from chewing the wires. The wires used (30-gauge) were nylon-wrapped iron and constantan; both were contained in a flexible bundle only 1 mm. in outside diameter. Junctions were soldered and covered with deKhotinsky cement. In each circuit two cold junctions (to copper) were surrounded by ice and water; the potentials in each thermojunction circuit were automatically balanced and registered by a recording electronic potentiometer. Successive readings were accurate to ±0.1°C.; however, during 24 hours the calibration changed by ±0.3° on the average. Hence, for most purposes, calibrations were made daily. As the calibration shifted, however, the increment of potential per degree of temperature did not change.

Course of Cooling. Colonic temperatures diminished with time in a highly reproducible fashion (fig. 1). A slight lag, lasting less than two minutes, was followed by a precipitous fall and by a final approach to an asymptote which approximated the water temperature. In rats of 200-gm. body weight, immersed to the shoulders, half the difference between initial colonic temperature and water temperature was traversed in about 13 minutes, regardless of the water temperature, provided the latter did not exceed 20°C.

The final difference of temperature between colon and medium was largest when the body's tissues were at 34° (fig. 2). Evidently this gradient in a warm rat can amount to 4°C. in a tissue thickness of only 2 cm. or less. This large gradient resulted from at least two factors: a rapid production of heat which was greater than at neutral or usual body temperatures (38°) and great internal insulation, secured by

Fig. 1. COURSES OF COLONIC TEMPERATURE in rats of 200 to 260-gm. body weight when immersed to the shoulders in water of diverse temperatures. Each curve is the mean of 5 individuals exposed at one time.
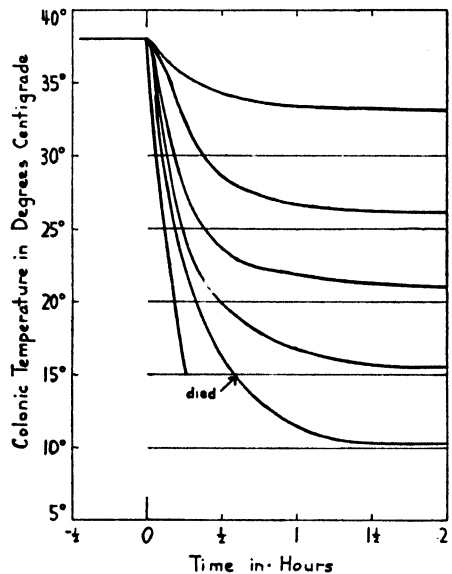
minimalization of blood flow. These factors were illustrated by comparing the cooling curves of living rats and dead rats (fig. 2). In contrast, when the rats were in water of 37° the heat production was low, the insulation being dissipated by rapid circulation of blood to the body surface. At any one time after immersion, colonic temperatures tended to be linearly related to water temperatures (fig. 3). Compensations successfully combatted this tendency only in the region of 30° water temperature. In water of 20° and below, the difference of temperature between colon and medium at the end of two hours was less than 1°C. Esophageal thermocouples indicated that the chest region also had similar temperatures (table 1).

When rats were immersed only to the hips, cooling was considerably slower (fig. 4). Moreover, the anterior regions of the body could now be considerably warmer than the posterior (table 1), by as much as 4°C. Seemingly the blood flow to the cooled posterior regions diminished to a point where little heat was carried to them.

When the rats were enclosed in rubber jackets that were held some distance from the body surface, cooling was very greatly delayed (fig. 4). The insulating effect of an air layer next to the skin is well known and its remarkable protection was here illustrated. In 12° water the rat immersed to the shoulders died in less than one hour;
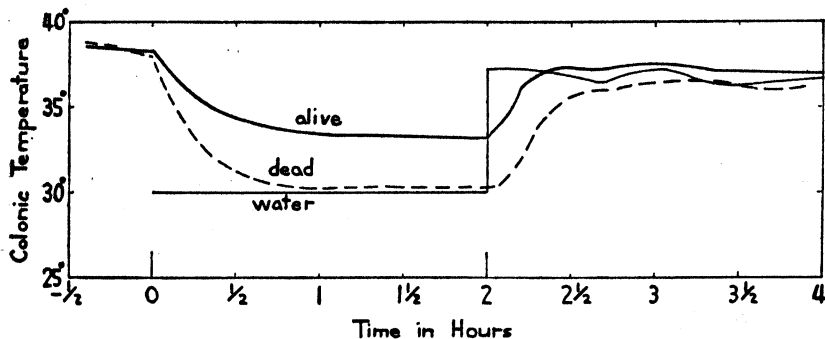

Fig. 2. COURSES OF COLONIC TEMPERATURES in rats of 225 gm. each, immersed in water o 30° and then transferred to water of about 37°. The solid curve represents the mean of 4 living rats he dash curve the mean of 2 rats previously killed by placing them in nitrogen for 3 min.
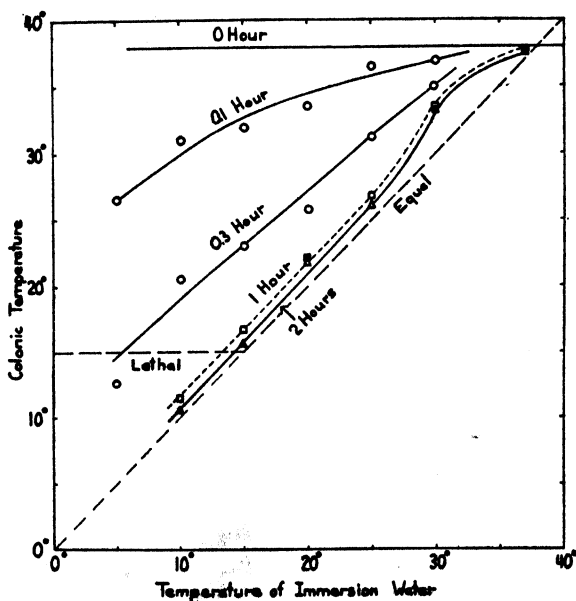
Fig. 3. COLONIC TEMPERATURES in relation to water temperatures surrounding the rats. Points were read off from fig. 1. Survival is usual within the area enclosed by dash lines.

the rat immersed to the hips might survive for 8 hours; and the rat in a jacket would hardly be in danger of death.

Lethality. Each standard test of ability to survive was conducted by immersing to the shoulders 5 rats, of about 200 gm. each, for two hours in a large water bath that was held constant within ±0.1°C. Subsequent rewarming was usually 'spontaneous' in air of about 25°C. and after three hours of it the animals were unbound and returned to cages. Those alive at the end of 20 or more hours were found to survive indefinitely.

The mortality curve was established on 90 control rats (fig. 5), about half of which died in two hours. By interpolation, the median lethal temperature of the water was 14.8°C. The lethal colonic temperature cannot be established quite so

TABLE 1. SIMULTANEOUS TEMPERATURES IN ESOPHAGUS AND IN COLON OF RATS
COOLING WHILE IMMERSED IN WATER

IMMERSION	WATER TEMP.	DURATION	FINAL COLONIC T.	FINAL ESOPH. T.	EXPIRED AT	COLONIC T. THEN	ESOPH. T. THEN
	°C.	hr.	°C.	°C.	hr.	°C.	°C.
in jacket	12.0	6.2	12.4	15.0	3.2	15.6	17.5
to hips	10.0	5.0	11.7	15.7	night		
	10.0	5.0	11.9	14.8	night		
to shoulders	20.0 to 11.8	5.0	12.0	12.3	2.5	14.5	15.2
	13.4 to 11.8	2.0	11.9	11.9	0.9	13.8	13.1
to hips	12.0	5.0	12.1	14.9	3.1	12.6	14.9
	12.0	5.0	12.2	15.0	5.2	12.4	15.2
	10.0	3.0	10.9	14.7	1.7	10.9	14.9
	10.0	3.0	12.5	16.2			
	10.0	3.0	11.9	16.9			
	10.0	3.0	10.4	11.8	2.3	10.5	13.3
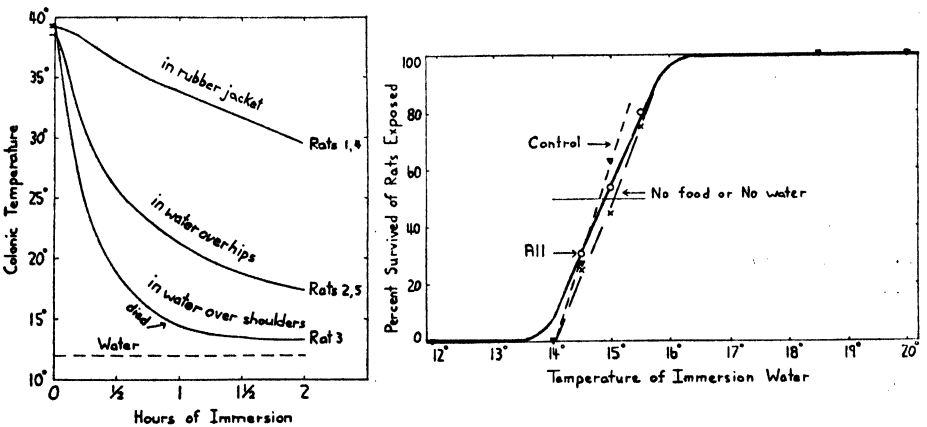
Fig. 4 (*left*). COURSES OF COOLING in rats treated in 3 ways in one water bath at 15°C.
Fig. 5 (*right*). FRACTION OF RATS that survive when for 2 hours they are immersed to the shoulders in water of the temperatures indicated. The numbers tested are: 15 at 14.0°, 35 at 14.5° and 50 at 15.0°, 5–10 at each other point. Some of the treatments, as in table 2, are indicated to show that subsequent differences in survival are not significant.

accurately, since during these tests the calibrations of the thermojunctions varied by as much as 0.3°C. greater than the water temperature at the end of two hours immersion to the shoulders, making the mean lethal colonic temperature 15.1°C. A roughly similar lethal temperature was reported by Hamilton (4, 5) for unanesthetized rats that were cooled by immobilization in cold air.

In rats so immersed, breathing became uncertain, irregular and slow as the animals cooled. It could not be affirmed with certainty, however, that breathing wholly ceased in any individuals that survived. Heart beats continued in all survivals in which electrocardiograms were taken. Reflex movements of the head, and wink responses to mechanical irritation of the eye-lids, persisted in nearly all individuals that recovered. However, in 13 rats it was specifically ascertained that wink reflexes disappeared, yet the individuals recovered later. The time of immersion at which the wink reflex disappeared is shown in figure 6 for each individual that succumbed of those represented in figure 5. At each of four temperatures the median times for this 'death' are indicated. In water of 14.5°, 30 per cent of the animals recovered and survived; in 15.0°, 65 per cent survived.
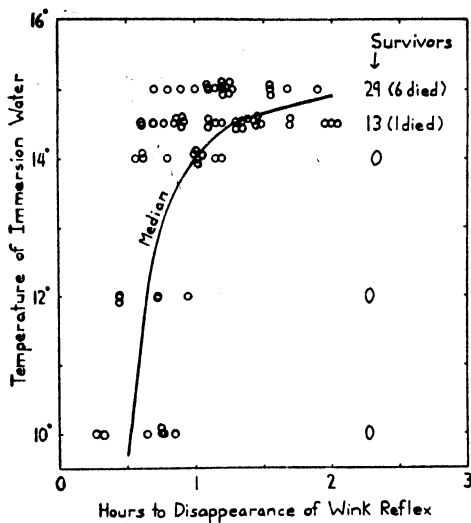
Fig. 6. TEMPERATURE of immersion water in relation to time before loss of wink reflex. At the end of 2 hr. rats were removed from the cold water and allowed to warm slowly; those that had not lost the reflex are designated as survivors, but of them 7 more died within 24 hr.

The body weights of the animals that succumbed before the median time were no less than those of the animals that lasted longer. However, other rats of less than 100-gm. body weight succumbed sooner; they are not included in this series. In such small individuals the cooling was more rapid, and slightly greater.

Variability. The mortality curve of figure 6 indicates that some individuals were more tolerant than others. Differentiation of this curve would show a frequency distribution of the lethal temperatures. Two thirds of the individuals would be included in a range of lethal temperatures within ±0.5° of the median. This therefore is the standard deviation among lethal water temperatures for two-hour exposures. That the variability of lethal effects was in time as well as in temperature was shown by the disappearance of reflexes (fig. 6). Once the reflex wink had faded, survival had become improbable.

Some of the variability was within the individual. In one instance a rat that survived in water of 14.5° succumbed a week later during immersion in water of 15.0°. But no regular effect of previous subjection to cold immersion was found, providing several days intervened between exposures. Part of the variability was prob-

ably in the treatment of the rats. It was not possible to equalize the exact extents of immersion, the bodily positions (which are concerned in struggling) and the stirring of the water. None of these factors was clearly proven to matter, however.

Detailed study of single individuals that were cooled while surrounded by rubber jackets showed marked diversities of pulse rates (ascertained from electrocardiograms) at any colonic temperature that might be chosen. The heart was seen to beat at a colonic temperature as low as 13.8°, with subsequent recoveries of the animals after the wink reflex had temporarily disappeared. The heart could beat as infrequently as 20 times per minute (400 beats/min. at 38°). It could beat irregularly, often with partial block, and still recover, but it has not been seen to recover after cessation of as long as 10 seconds. During the rapid cooling and rewarming of these tests, the temperature of the chest might differ appreciably from that of the colon.

Various Rates and Durations of Cooling. The rates of cooling of rats were varied by *a*) using water of diverse temperatures from 5° to 15°; *b*) immersing the rats to diverse extents; *c*) surrounding the animals with rubber jackets; and *d*) gradually cooling the water in which the rats were immersed to the shoulders. From all these experiments the conclusion was drawn that lethal injury resulted whenever the tem‌perature of the anterior half of the body had decreased to 14° to 15°. The period of time (up to 6 hr.) during which the body remained at this temperature mattered chiefly in the fact that disappearance of reflexes and other functions caught up with more individuals. For the animals immersed only at the posterior end, the colonic temperatures could be 3 to 4°C. lower than the esophageal temperatures (table 1). No attempt was made to find whether the chest or the head was the critical location for the low temperature.

Whether the lethal damage occurred in a particular tissue, or in several, was uncertain. The heart beat, the breathing and the eye-wink reflex all slowed as the temperature of the animal fell and tended to reach zero at 13° to 15°C. While the continuance of these functions could not always be taken as guaranteeing survival, nor their cessation as preventing it, the functions were probably critical ones. Similarly, it is not implied that colonic temperature was the only factor determining survival. This report is not specifically concerned with identifying the modes of death, but with establishing a reproducible test of lethality in cold, against which the protective power of diverse procedures might be tested.

Present evidence points to the conclusion that the *duration* of the lethal tissue temperature (15.1° ±0.5°) was of little importance. In five tests, individuals were rapidly cooled and then quickly rewarmed, so that their colonic temperatures dipped below 15.0° for only 6 to 18 minutes. However, two of them were killed just as were individuals maintained at 14.7° for two hours. On the other hand, continuance of the cold immersion up to five hours at 15° was not significantly more lethal than for two hours. However, for 12 hours, water of 16° came to kill the rats immersed in it.

Possible Influences upon Survival in Hypothermia.

a) *Age.* Rats of all ages above 26 days died at the same average body temperatures when immersed in cold water. In the series of experiments so far quoted, animals ranged from 1½ to 12

months in age, and 103 to 360 grams in weight. Other individuals of 26 and 27 days of age (40–49 grams) were tested for 2 hours; all in 14° water died; three-fifths survived in 15° water. Hence from 26 days upward no differences due to age or size were found, providing the chest temperatures attained were brought close to the water temperatures. Only rats in earlier infancy survived lower body temperatures.

b) Sex. Equal numbers of males and females were used in the main series. No difference of mortality was found between them.

c) Season. No differences of lethal temperatures were found among series of tests done in each quarter of the calendar year.

d) Rate of rewarming. Most of the rats exposed to cold were allowed to rewarm in air of 25°. More rapid rewarming occurred in air of higher temperature usually 30°; still more rapid in water of 35°. Survival of the rats differed only to the small extent that some were incompletely rewarmed before they were returned to their food cages, causing delayed death (see below).

e) Deprivation of food. Individuals were kept without food but with water for 2, 3, or 4 days before they were chilled. The difference in survival was scarcely significant (fig. 5 and table 2); and survival was less than in controls, a result opposite to that noted by Fuhrman and Crismon (6) in anesthetized rats.

f) Deprivation of water for similar periods of time likewise did not favor survival. It may be remarked that privation of either food or water induced self-denial of the other item of intake (7).

g) Glucose was administered, usually 1.5 gm. by stomach tube, to 11 rats. The proportion of them that survived chilling was no different from that of simultaneous controls (table 2). In anesthetized rats, administration of glucose just before cooling upon chilled metal, irregularly delayed the stoppage of heart and breathing movements (6).

h) Adrenal cortical hormone (cortin) was injected subcutaneously (0.5 or 1.0 ml. Parke Davis), with no deviation in lethal effect from that of the controls. This result also contrasts with that of the above investigators.

i) Oxygen around head. Rats that were being cooled by immersion to the shoulders had their heads covered by a rubber cowl, into which passed a continuous stream of oxygen. By this means their lungs were left filled with an atmosphere of oxygen whenever they stopped breathing. In contrast to the positive result in infant rats, no greater survival resulted here (table 2).

j) Digitalis. Equal numbers of tests were made after intraperitoneal injection (at 20–60 minutes before chilling) of each of the following: 0.67 ml. digalen, 0.17 ml. digalen, 0.60 ml. cedilanide (or lanatoside C). Mortality in water of 14.5° did not decrease, contrary to expectation from the results of Crismon and Elliott (8) but not from those of Barbour *et al.* (9).

k) Acclimatization. Rats were acclimatized to cold by placing them with adequate food and water in air of about 7°C. for periods of 11, 17 or 30 days. If any treatment would make animals more tolerant to cold, this might well be it. But, no more survival was found than in control rats. Acclimatization may be considered as occurring in two possible ways: by change of resistance to cooling (by more production of heat or by less loss of heat or by both) and by change of tolerance of low temperatures within the tissues. In the present tests all resistance to cooling is rapidly overborne. Tolerance of cold is alone being tested; such tolerance has not been successfully modified.

The test of survival here employed is a sensitive one. In all cases the colonic temperatures of the treated rats did not differ by more than a few tenths of a degree from those of the control rats. However, the colonic temperatures that could be tolerated at the end of two hours of immersion up to the shoulders varied by a standard deviation of ±0.5°C. No treatment was found that lowered the median lethal temperature by even 0.2°C. Hence it may be concluded that the temperature, at which the tissues that limit a rat's survival are injured, is fixed to such an extent that it has not been modified by any of the influences tried.

The hibernating individual and the infant are the two kinds of mammals known whose *tissues* come to survive temperatures that are otherwise lethal. The hiber-

nating animal continues its breathing and blood-circulation at very low tissue temperatures (1°C.); only the infant survives (for a couple of hours) without either.

Delayed Death. In 21 rats exposed to cold apparent recovery began, only to be followed some hours later by death of the animals. These rats generally failed to regain their usual body temperatures 'spontaneously'; artificial warming would probably have saved them. Such deaths occurred occasionally in two or four hours after rewarming began, but more often happened during the night after an immersion. None occurred more than 24 hours after emersion.

TABLE 2. MORTALITY OF TREATED AND CONTROL RATS IMMERSED TO THE SHOULDERS
FOR TWO HOURS IN WATER OF CRITICAL TEMPERATURE

TREATMENT	WATER TEMP.	NO. TREATED	PER CENT SURVIVED	NO. OF SIMULTANEOUS CONTROLS	PER CENT SURVIVED
e. No food..............	15.0°	14	43	9	89
f. No water.............	15.0°	12	50	6	100
g. Glucose..............	14.5°	11	55	8	50
h. Cortin...............	14.5°	6	50	4	50
i. Oxygen at head.......	14.5°	10	20	5	80
j. Digitalis.............	14.5°	18	33	12	58
k. Acclimatized..........	14.5°	15	47	10	60

Fig. 7. COURSES OF REWARMING in room air and in warm water. Each curve represents the mean of 2–5 individuals.
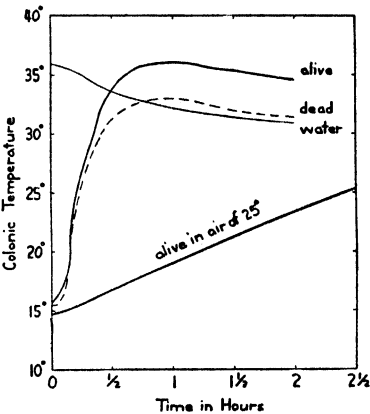
It seems probable that the lethal damage was inflicted during the period of low body temperature. No observed signs allowed prediction of the imminence of death; on the average these animals warmed more slowly and showed poorly coordinated locomotion. Where the lethal injury lay is unknown, but the central nervous tissue is naturally suspected. This form of delayed death was less critical than that found in heat stroke (10). In the latter, rats died up to two hours after they were cooled from a critical hyperthermia produced in a hot atmosphere, but both heat injury and cold injury may become irreversible some time before circulation and breathing actually cease. Delayed deaths have also been observed during 'recoveries' from food privation and from water privation.

At autopsy, rats that were killed in acute hypothermia or that died after hours of delay showed but little uniform abnormality. The lungs were congested to varying degrees; the congestion appeared to be uniform over the lungs of most individuals, but occasionally involved localized hemorrhages. Consolidation of the lungs has been sometimes observed but may have preceded the hypothermia.

Course of Rewarming. The progress of rewarming was surprisingly slow in rats. On the average they required 52 minutes to warm by the first 2°C. after emersion into air of 25°. The rewarming was steady (fig. 7) and became somewhat more rapid as the animals became warmer and resumed movements. The colonic temperatures of dead individuals leveled off, with the air temperature as asymptote. One fourth of the recovery of the 200-gm. living rat (from the minimal body temperature to the body temperature that preceded immersion) required 82 minutes, one half of it required 145 minutes. The latter could be reduced to 20 minutes by immersing the rat at once in water of 30°C.

The rate of rewarming in air manifested the animal's inherent abilities to recover after its body temperature had been disturbed. Evidently a rat cooled to below 20°C. was almost paralyzed in its recovery. Heat production was exceedingly small, reflexes had partially disappeared, circulation of blood was slow. Even when free movements had been recovered, shivering was small; rats do not have violent shivering as a part of their armamentarium of compensation for cold.

The chief effect of artificial rewarming was that it prevented the deep temperature from falling still lower after the animal was removed from water that was colder than the colon; when the rat had been quickly cooled in water of 5° to 10°, cooling by an additional degree or two could occur after emersion. In such a case rapid rewarming might be of vital consequence, but once temperature equilibrium had been established between body and water, the temperature did not diminish after the animal was emersed, for no evaporative cooling then occurred. Evaporation was already minimal, because the body was cooler than the air and the air was nearly saturated with moisture; the body might even gain heat by condensation of moisture.

<div align="center">COMMENT</div>

The above experiments measure the lethal effects of cold in terms of the temperatures to which tissues are subjected and from which they can recover. It is shown that the mature rat is killed whenever the temperature of its head and chest is decreased to 15.1°C. ±0.5° (standard deviation). The variability of tolerance in the tissues of individuals is therefore small indeed.

No method was found of modifying the lethal temperature. Plainly, tolerance of tissues to low temperatures is fixed. Modifications in resistance that are known to occur in mammals generally, therefore, belong to the processes or properties that oppose tissue cooling, but cooling is exceedingly fast when a small mammal is immersed. The only exception in tissue susceptibilities is the spontaneous transformation of some mammals into hibernating ones. The rat's measures for protection all fall into the class of provisions that will delay cooling. Artificial provisions will evidently fall into the same class. Immersion in cold water furnishes more rapid cooling of a rat than any other means that has so far been tested. It yields more uniform cooling than is attained in air or other media. It provides a quick test of

tissue tolerance to cold. Further, it shows that survival temperatures of tissues are the same whether the rat be cooled slowly or rapidly. Over longer periods of time than the 2-hour and 5-hour periods that we have used, time might become a factor in survival, as was shown to be the case in guinea pigs (1). In the present experiments a period of four hours at 15° colonic temperature killed few more rats than a period of one hour; while a period of 12 hours at 16° was lethal. The percentage of delayed deaths may or may not be greater in the prolonged immersions.

Simultaneous measurements of esophageal and colonic temperatures in rats showed that during partial immersion in water of 12°C., a temperature gradient of 3 to 4°C. can be maintained between anterior and posterior portions of a rat as small as 200 gm. In that water the gradient makes the difference between death and life. Such a gradient can exist transversely in this small animal only when the temperature of the body is very high (above 30°). At low temperatures the rate of heat production is too low to support it. The lethal temperature during water immersion is surprisingly constant among individuals and with age above 26 days after birth. Moreover, no treatment has yet been found to influence the ultimate survival. Many other treatments need testing in the hope that some will prove effective.

SUMMARY

Median lethal temperatures were ascertained by immersing mature rats for 2 hours in waters of constant temperatures. The median lethal temperature of surrounding water was 14.8°C., its standard deviation was ±0.5′. The colonic temperatures were ordinarily 0.1° to 0.3° higher than the water temperatures. Rates of cooling were not significant factors in the survival of rats after lethal cooling. Sometimes after incomplete rewarming, death was delayed for many hours after apparent recovery. No factor was found to modify the lethal temperature of the unanesthetized rat. Age above one month, season, body size, sex; administrations of glucose or cortin, digitalis or oxygen; deprivations of food or of water; acclimatization to cold air, all failed to modify the lethal temperature. Resistance to cold in rats appears not to be modifiable, therefore, in the tolerance of tissues, but depends solely upon delays in the cooling of essential tissues.

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INFLUENCE OF TEMPERATURE ON THE RESPONSE OF FROGS TO X IRRADIATION

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IN A preliminary note, we reported that low temperature treatment of frogs after total-body exposure to x-rays greatly prolongs their survival (1). The present studies were initiated to determine: 1) whether this altered radiosensitivity is due to a decrease in the rate of development of radiation injury or actually reflects a more rapid and complete recovery from the effects of irradiation and 2) whether body temperature during exposure influences the radiation reaction. Since the overt response of the frog, a poikilotherm, to x-radiation appears to be rather similar in many respects to that observed in the mammal, it was thought that these studies might be of practical as well as theoretical interest.

METHODS

Male frogs (*Rana pipiens*) weighing 20 to 30 gm. were housed in stainless steel or glass aquaria provided with platforms to enable them to remain in or out of water. The stock animals were kept in a constant temperature room (22°–23°C.) and were not fed during the experiment. Each shipment of frogs was observed for 2 to 4 weeks before irradiation and those animals obviously in poor condition were discarded. In spite of all precautions, some deaths occurred from time to time among the stock animals. To control this spontaneous mortality, nonirradiated frogs were observed along with their irradiated members in each experiment. Moreover, in each experiment the various conditions of temperature were evaluated on groups of animals received at the same time.

Frogs were irradiated in a circular cell of perforated aluminum divided into 10 radially arranged individual compartments. The exposure box was rotated slowly on an electrically driven turntable to assure equal irradiation of all the animals. The radiation factors were: 200 kv.; 15 ma.; 0.5 mm. Cu. and 3.0 mm. bakelite filters; target distance 45 cm.; dose rate 45–50 r/min., total dose 1000 to 9000 r.

For irradiation at different body temperatures, the exposure box was fitted into the inner chamber of a cylindrical constant temperature cell. Water or ice was placed in the concentric outer chamber to achieve the desired ambient temperature. Except for the lucite top of the inner chamber through which the x-ray beam was directed, the entire cell exterior was insulated with rock wool. Air temperature of the inner chamber did not vary by more than ±1°C. from the beginning to the end of an exposure. Frogs were placed in the constant temperature exposure cell two hours before irradiation. Rectal temperature measurements made with copper-constantan thermocouples revealed that this interval was sufficient to assure body and ambient temperature equilibrium, with the former usually some few tenths of a degree centigrade above the latter. Animals which were kept at low temperatures after x-irradiation were placed in large refrigerators equipped with special thermostats.

Survival was the major criterion of radiation effect. In a pilot series, the radiation-induced changes in blood cell counts and in the histopathology of certain tissues were also compared under different conditions of temperature. Hematological data were obtained in frogs given 3000 r at 23°C. subsequently kept at 5°C. for 28 days and then returned to 23°C. Counts were made at

weekly intervals on three frogs in each experimental group. Comparison was made with appropriate controls.

RESULTS

Toxicity data for animals maintained at different ambient temperatures after exposure to various roentgen doses delivered at 22° to 23° C. are summarized in table 1. The prolonged survival with low-temperature treatment is clearly evident even for a radiation dose (9000 r) which is almost nine times the completely lethal dose. Some 80 to 90 per cent of the animals kept at 5° C. for three to four months after irradiation survive a dose (3000 r–6000 r) which kills all frogs at 22° C. within two to five weeks. However, when the former are removed from the cold even after

TABLE 1.　INFLUENCE OF TEMPERATURE UPON SURVIVAL OF FROGS AFTER X IRRADIATION[1]

DOSE, r	TEMPER-ATURE, °C.	NO. OF FROGS	PERCENTAGE SURVIVAL—WEEKS AFTER X IRRADIATION									
			1	2	3	4	5	6	7	8	13	18
1000	23	38	97	95	82	58	27	19	19	13		
1000	6	20	100	100	95	95	90	90	90	90		
3000	22	129	95	79	32	5	0					
3000	12	10	90	80	70	70	70					
3000	5	58	100	100	98	96	96	94	92	92	80[2]	
6000	23	12	100	0								
6000	5	12	100	100	100	100	100	100	100	100	100	100
9000	22	12	100	0								
9000	5	12	100	100	100	100	75	42	0			

[1] All animals were irradiated at 23°C.　　[2] Only 30 of the original group of 58 animals were observed at 13 weeks.

prolonged periods, death ensues. The time for 50 per cent lethality at 23° C. after varying periods of low-temperature treatment is presented in figure 1. It will be noted that ultimate toxicity is not influenced significantly by keeping frogs at 5° to 6° C. for 60 days after 1000 r, 75 days after 3000 r and 130 days after 6000 r. Mortality of the nonirradiated temperature controls averaged only 5 to 10 per cent over these intervals. Inspection of figure 2 reveals that the survival curve for the 1000 r frogs after their removal from low temperature is identical with that obtained for similarly exposed animals without cold treatment. Similar results were observed in the 3000 r and 6000 r groups.

The ambient temperature at which frogs are x-irradiated is clearly without effect on survival (fig. 2). Similar responses are obtained in the groups exposed to 1000 r at 1° to 3° C. and at 21° to 23° C. Combining low temperature during exposure to 1000 r and for the first 24 hours after exposure likewise does not influence toxicity. Percentage survival and the mean survival time are also not altered appreciably when frogs are irradiated with 3000 r at 5° C., 12° C., 21° C. and 32° C. (fig. 3).

When the blood counts of irradiated frogs are expressed as percentage changes from their respective temperature controls, no definite trends are discernible in red cell number. Blood platelets tend to decrease (6th day), then increase (13th and

2oth days) after irradiation. These changes seem less severe in the animals kept at low temperature after exposure. However, the clearest indication of a tempera-
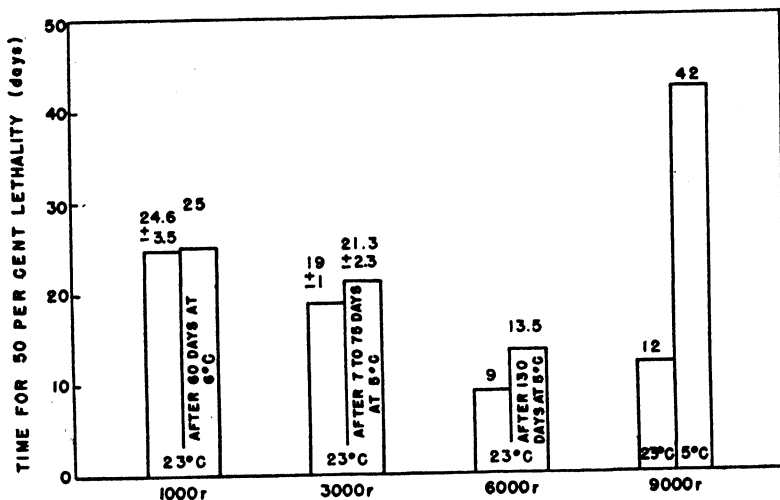

Fig. 1. EFFECT OF LOW TEMPERATURE TREATMENT upon survival of x-irradiated frogs after their removal to 23°C. (All animals irradiated at 23°C. Period of cold treatment not included in calculations of 50% survival time except for the 9000 r group in which deaths occurred at low temperature.)
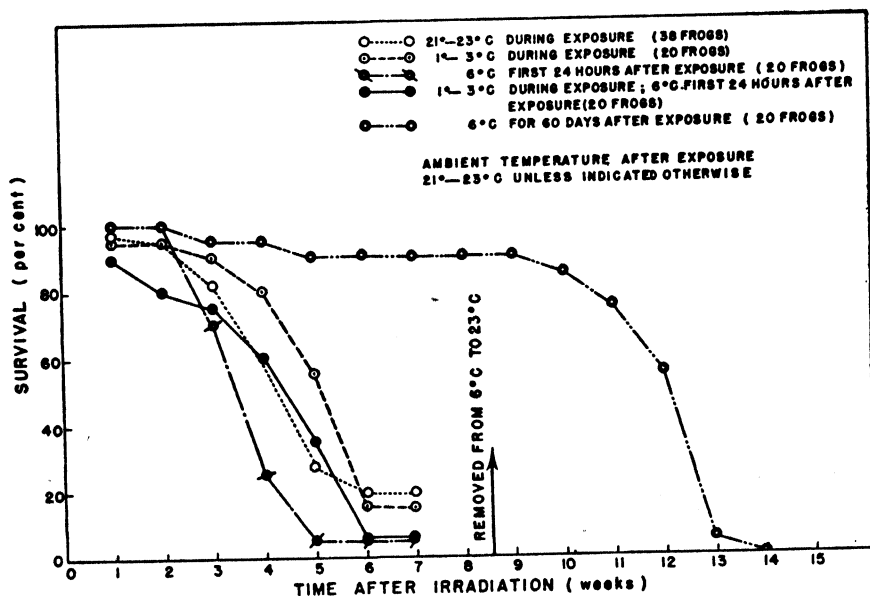

Fig. 2. EFFECT OF TEMPERATURE upon survival of x-irradiated frogs (1000 r).

ture effect is in the total white cell count, which decreases to a lesser extent in the irradiated frogs kept at low temperature. Yet, when the cold-treated animals are returned to room temperature after 28 days, their leukocyte count is depressed and reaches a low level comparable to that seen in the nonrefrigerated group (fig. 4).

Histological studies on frogs sacrificed at 2, 4, 6, 24 and 48 hours following exposure to x-rays at 23° C. reveal that cellular degeneration of lymphocytes in the spleen and intestinal submucosa is evident at 2 hours in animals kept at 23° C. and at 48 hours in those at 5° C. There is apparently no further damage after 6
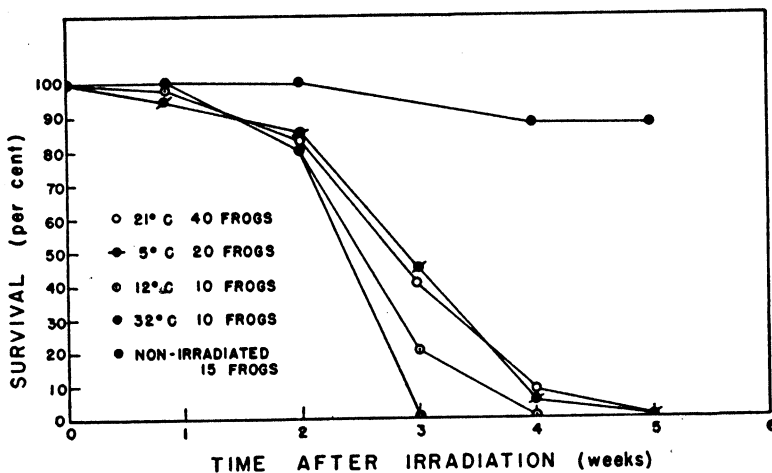
Fig. 3. EFFECT OF TEMPERATURE during x-irradiation on survival (3000 *r*).
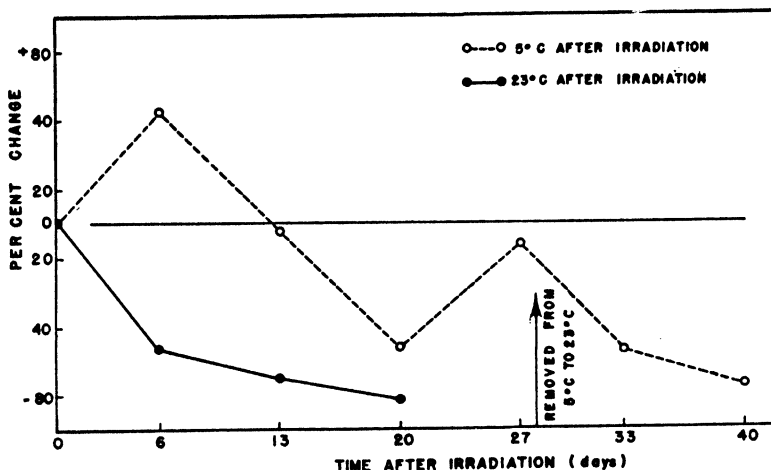
Fig. 4. INFLUENCE OF TEMPERATURE on leucocyte count of irradiated frogs (3000 *r*).

hours in the animals kept at room temperature, and at 24 and 48 hours there is no appreciable difference in these animals from the nonirradiated temperature controls.

DISCUSSION

There is evidence which suggests that the radiosensitivity of tissue may be dependent upon certain aspects of its activity. An increase in growth rate, cell division, respiratory activity, blood flow etc., induced by temperature, drugs and other

experimental manipulation, is said to enhance susceptibility to radiation under certain conditions (2–8). On the other hand, a change in cell environment can also alter the response to ionizing radiation independently of a change in the number of dividing cells and of oxygen consumption, or can even fail to influence sensitivity though growth rate and metabolic activity are greatly diminished (5, 9).

The eggs of *Ascaris* and *Drosophila* and the skin of young rats irradiated at low ambient temperature are more resistant than those at high temperature (2, 4, 6). The reverse is true for the broad bean root and for mouse tumor tissue (9, 10). Radiosensitivity of wheat seedlings and of thymic cell suspensions is not affected by low temperature (5, 11). When chick embryos and *Ascaris* eggs are maintained at low temperature after exposure, radiation injury is delayed in the former and apparently repaired in the latter (12, 13). Similar experiments with the broad bean root, *Ascaris* eggs and thymic cell suspensions, in which desiccation, an oxygen-poor environment and low temperature respectively were utilized to decrease cellular activity after irradiation with gamma and x-rays, reveal that there is no appreciable recovery under these conditions (14, 11). Many of these findings are difficult to reconcile solely on the basis of differences in the test objects and in the criteria of radiation effect employed and are perhaps indicative of the complexity of radiation reactions.

Survival of frogs is not influenced by altering their body temperature during and/or for the first 24 hours after x-irradiation. Similarly, we noted in other experiments that toxicity was unchanged when the oxygen consumption of frogs was depressed by some 50 per cent during exposure to x-rays as a result of a prior injection of potassium cyanide (15). Survival is greatly enhanced, however, as long as the frogs are kept at low temperature continuously after irradiation. Altered sensitivity in the cold is due apparently to a decrease in the rate of development of radiation damage (prolongation of the latent period) rather than to any appreciable recovery. When the animals are removed from the cold after a prolonged period, there is no change in absolute survival nor any clear difference in the time course of deaths from that observed in irradiated animals maintained at 23° C. Additional evidence in support of this interpretation is presented in the hematologic and histologic findings in the few frogs in which these determinations were made.

We may conclude from these studies in frogs that the primary reactions occurring during exposure to ionizing radiation, which initiate the changes leading to morbidity, are independent of body temperature over a wide range and are apparently physical or photochemical in nature. The changes incidental to irradiation (secondary reactions) are, however, temperature sensitive. Yet, modifying the rate of these secondary reactions by depressing body temperature for prolonged periods is without effect on the final outcome. This suggests that the mechanisms concerned with recovery may, likewise, have a high temperature coefficient.

<div align="center">SUMMARY</div>

Toxicity is not influenced by altering the body temperature of frogs during and/or for the first 24 hours after total-body x-irradiation with 1000 *r* and 3000 *r*. Survival is greatly enhanced, however, as long as the animals are kept in the cold (5°–6° C.)

continuously after the exposure. This altered sensitivity is due apparently to a decrease in the rate of development of radiation damage (prolongation of the latent period) rather than to any appreciable recovery. When the animals are removed from the cold after periods of 60 to 130 days, there is no change in absolute survival nor any clear difference in the time course of deaths from that observed in irradiated animals maintained at 23° C. These findings indicate that the metabolic level during exposure does not influence the overall radiation reaction in frogs and that the primary process of radiation damage is not repaired in the metabolically depressed animal.

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AN ALTERED PROTHROMBIN PRODUCED BY DICUMAROL-TREATED RABBITS[1]

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SCHOFIELD (1) was the first investigator to describe the hemorrhagic disease of cattle known as sweet clover disease. The work of Roderick (2, 3) indicated that the increased clotting time was primarily due to a deficiency in the prothrombin content of the blood. The causative agent of this disease was isolated by Campbell and Link (4) and the chemical studies of Stahmann *et al.* (5) showed it to be 3,3′-methylenebis(4-hydroxy-coumarin) known popularly by the trade name of Dicumarol. Further work with Dicumarol tended to confirm the hypothesis that the substance prevents the synthesis of prothrombin in the liver (6–9). The evidence for this view has been obtained by determining the clotting time of plasma from treated and untreated animals using excess protein thromboplastic agents and optimal quantities of calcium ions. The clotting time of the animals treated with Dicumarol was markedly increased and it was thought that this indicated a lack of prothrombin since the other necessary clotting factors were present in excess.

During a study of the effect of Dicumarol on the prothrombin content of dog plasma, it was noted that clotting of such plasma occurred more rapidly with a lipid thromboplastic agent than with a protein thromboplastic agent. Since it has been frequently shown that the protein thromboplastic agents are more active than the lipid ones (10, 11), this anomalous behavior was investigated.

METHODS

Reagents

Lipid thromboplastic agent. This agent was prepared from beef brain by the procedure of Hays and Lein (11). It consists of a mixture of phospholipids and gave no evidence of containing protein by the xanthoproteic test. The material was protected from autoxidation by shaking with a saturated solution of hydroquinone in acetone, collecting on a Büchner funnel and drying *in vacuo*. This material showed an optimal thromboplastic activity at a concentration of 3 mg/ml. of 1 per cent NaCl. An emulsion of the lipid at this concentration was prepared weekly and kept at 5°C. when not in use.

Protein thromboplastic agent. Beef brain served as a source of this agent. The brains were stripped of their meninges and macerated in two volumes of 1 per cent NaCl. The mixture was placed at 5°C. for 20 hours and then strained through cheesecloth. The liquid was centrifuged at 2000 r.p.m. for 30 minutes and an equal volume of saturated ammonium sulfate was added to the supernatant. After 30 minutes at 5°C. a flocculent precipitate settled out and was packed by centrifugation. The tan residue was dissolved in one per cent NaCl, one part of saline to six parts of the original weight of brain tissue. This protein thromboplastic agent was stable over a period of at least one month. The small amount of ammonium sulphate present in the preparation was not removed by dialysis. Experiments on the clotting of diluted plasma by the protein agent indicated that the concentration of ammonium sulphate was too small to have an inhibitory effect under the conditions in which the protein agent was used.

Fibrinogen. The fibrinogen was prepared from beef blood by the method of Jacques (12). This method consists essentially of precipitating the protein three times in a 1M phosphate solution of pH 6.6 and dialyzing it to get rid of the phosphate.

Prothrombin. Two types of prothrombin preparations were used. One, derived from beef blood, was kindly supplied by Dr. Walter H. Seegers. It was a very highly purified prothrombin obtained by the method of Ware and Seegers (13). The preparations of prothrombin from rabbit plasma were made using the procedure of Munro and Munro (14). This method is based upon the adsorption of prothrombin on aluminum hydroxide and its elution with a 0.2M phosphate buffer of pH 8.0.

PROCEDURE

Experiments were carried out using rabbit plasma. Blood was drawn by cardiac puncture directly into 0.13M sodium citrate, using one part of citrate to nine parts of blood. The blood was immediately centrifuged at 2000 r.p.m. for 25 minutes and the plasma removed. Clotting times were determined in Pyrex tubes, 10 x 75 mm., using 0.6 ml. of plasma, 0.2 ml. of thromboplastic agent and 0.2 ml. of 1 per cent calcium chloride. After mixing the reagents the time in seconds when the tubes could be inverted without flow of contents was determined. This was considered to be the clotting time. These times were determined at least in duplicate and the mean values are recorded in the data. Clotting times were also determined with 1 per cent NaCl substituted for the thromboplastic agents. These mixtures clot due to some extraneous thromboplastic agent being present in the plasma. All experiments were carried out at room temperature.

RESULTS

Clotting of Dicumarol plasma. A total of 9 rabbits was used in the Dicumarol experiments. The clotting times were first determined before giving them Dicumarol. Dicumarol, 10 mg/kg., was then mixed with a small quantity of the rabbits' food, the bulk of the food being withheld until all the treated food had been consumed. The Dicumarol was given on three successive days and a blood sample was withdrawn on the fourth day. The animals showing prolonged clotting times invariably died. Autopsy revealed excessive bleeding into the pericardial cavity.

The plasma obtained from 6 of the Dicumarol-treated rabbits was divided into two portions. The clotting times were determined on one of these portions. To the other, excess purified beef prothrombin in the form of powder was added and the clotting times noted. This was done to determine if normal relationships would be restored in Dicumarol plasma by the addition of purified prothrombin. The clotting times in seconds of this series of experiments are presented in table 1.

The data of the table show that with normal plasma the protein thromboplastic agent is considerably more active than the lipid agent. In 7 of the 9 rabbits this relationship is drastically changed in the Dicumarol plasma. In 4 of these rabbits, *1, 2, 3* and *6*, the Dicumarol plasma did not clot with the protein thromboplastic agent in over 11,000 seconds while the lipid agent induced clotting in an average of 404 seconds. *Rabbits 5, 8* and *9* also showed markedly longer clotting times with

TABLE 1. CLOTTING OF NORMAL PLASMA, DICUMAROL PLASMA AND DICUMAROL PLASMA PLUS PROTHROMBIN BY SODIUM CHLORIDE, THE LIPID THROMBOPLASTIC AGENT AND THE PROTEIN THROMBOPLASTIC AGENT

	CLOTTING TIMES								
RABBIT NO.	Normal plasma			Dicumarol plasma			Dicumarol plasma plus prothrombin		
	NaCl	Lipid	Protein	NaCl	Lipid	Protein	NaCl	Lipid	Protein
1	186	70	57	1135	443	>11,000			
2	178	79	50	1620	420	>11,000	273	95	87
3	189	100	63	1425	293	>11,000	210	100	57
4	288	132	53	563	273	183			
5	223	91	63	1350	600	1,380	161	71	47
6	245	94	57	1785	460	>11,000	160	85	58
7	193	70	53	275	110	100			
8	327	110	53	1468	193	2,480	160	70	40
9[1]	305	77	39[1]	1560	263	3,600	180	63	38[1]

[1] A new preparation of the protein thromboplastic agent was used in this experiment.

the protein agent as compared with the lipid agent. *Rabbits 4* and *7* did not show these marked effects. Campbell *et al.* (15) showed that some rabbits are more resistant to Dicumarol than others and that the resistance is inherited in simple Mendelian fashion. It is thought that *rabbits 4* and *7* may be Dicumarol-resistant rabbits. Results also indicate some increase in the clotting time of Dicumarol plasma by the lipid. These values are invariably longer than those of the normal plasma. Adding highly purified prothrombin to the Dicumarol plasma caused the clotting times to revert to the condition found in normal plasma.

It is interesting to note in the data that the 7 rabbits showing the altered clotting relationship of protein and lipid thromboplastic agents also have a longer clotting time when protein thromboplastic agent is added than when one per cent NaCl is used. This indicates that the protein agent is actually inhibiting the clotting of the plasma by the thromboplastic agent normally present in the plasma. An experiment was carried out to see if the protein would inhibit clotting of the Dicumarol plasma by the lipid agent. Clotting time was determined on Dicumarol plasma of

rabbit 3 using in one case 0.6 ml. of plasma, 0.1 ml. one per cent NaCl, 0.1 ml. lipid agent and 0.2 ml. one per cent CaCl₂. The plasma clotted in 285 seconds. A second tube containing 0.6 ml. of plasma, 0.1 ml. protein agent, 0.1 ml. lipid agent and 0.2 ml. of one per cent CaCl₂ clotted in 660 seconds. This experiment shows that the protein actually does act as an inhibitor of clotting in Dicumarol plasma.

Physical reduction of prothrombin content. In view of the results obtained with Dicumarol plasma, experiments were carried out in which the prothrombin content of normal plasma was reduced by physical methods. The clotting times of such plasmas obtained with the lipid and protein thromboplastic agents would indicate whether the Dicumarol results could be explained on the basis of reduction of prothrombin content of the plasma or whether some other explanation would be necessary. The prothrombin in normal plasma was reduced in concentration by two methods. In the first the prothrombin was reduced by dilution and in the second it was reduced by adsorption on aluminum hydroxide.

TABLE 2.   CLOTTING OF DILUTED PLASMA BY LIPID AND PROTEIN THROMBOPLASTIC AGENTS

	CLOTTING TIMES			
% OF NORMAL PLASMA	Diluted with 1 % NaCl		Diluted with fibrinogen solution	
	Lipid	Protein	Lipid	Protein
100	61	52	41	39
50	86	58	45	41
25	105	70	54	49
12.5	130	106	99	90
6.3	220	143	135	109
3.1	315	285	276	175
1.5			435	294

Two samples of plasma were used for the dilution series. One sample was diluted using one per cent NaCl as the diluent while the other was diluted using fibrinogen solution. Clotting times were determined at the various dilutions using the diluted plasmas, thromboplastic agents and CaCl₂ in proportions described in the methods section. These results are presented in table 2.

It will be seen from the data of table 2 that when normal prothrombin is decreased in concentration by dilution, clotting by the protein thromboplastic agent is always more rapid than by the lipid. Even though the clotting time of the more dilute plasmas clotted by the lipid agent were of the same order of magnitude as that of the Dicumarol plasma of table 1, clotting times using the protein agent were still shorter.

The prothrombin concentration was also reduced in normal plasma by adsorbing the prothrombin on various amounts of aluminum hydroxide cream.[3] This preparation has been shown by Munro and Munro (14) to adsorb prothrombin. The aluminum hydroxide cream was added to normal rabbit plasma to make volume con-

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[3] The aluminum hydroxide cream used was Wyeth's 'Amphojel' without flavor. This was kindly supplied by Doctor Barol of Wyeth Corporation.

céntrations of 1, 2, and 3 per cent. It was mixed thoroughly and allowed to stand at room temperature for 15 minutes. After this time the aluminum hydroxide was centrifuged down and the plasma used for the clotting time tests. Clotting times were determined as before using the adsorbed plasmas and the protein and lipid thromboplastic agents. Clotting times with one per cent NaCl instead of the thromboplastic agents were also determined. The experimental results are presented in table 3.

The data of table 3 indicate that if one reduced the prothrombin content of plasma by adsorption on aluminum hydroxide, the protein thromboplastic agent still shows greater activity than the lipid agent.

Isolation of altered prothrombin from Dicumarol plasma. The above experiments indicate that reducing the prothrombin concentration by physical methods gives

TABLE 3. CLOTTING OF ALUMINUM HYDROXIDE-TREATED PLASMA BY SODIUM CHLORIDE, THE LIPID THROMBOPLASTIC AGENT AND THE PROTEIN THROMBOPLASTIC AGENT

| % Al(OH)₃ | CLOTTING TIMES | | |
	NaCl	Lipid	Protein
0	173	78	60
1	558	141	111
2	1020	190	135
3	1320	255	185

TABLE 4. CONVERSION OF PROTHROMBIN ISOLATED FROM NORMAL AND DICUMAROL PLASMA TO THROMBIN BY THE LIPID AND PROTEIN THROMBOPLASTIC AGENTS

| TYPE OF PLASMA | CLOTTING TIMES | | |
	NaCl	Lipid	Protein
Normal	195	95	85
Dicumarol	690	443	1005

different results in relation to thromboplastic clotting than does treatment of rabbits with Dicumarol. It was thought advisable therefore to attempt an isolation of prothrombin from plasma of a rabbit treated with Dicumarol and compare it with prothrombin isolated from a normal rabbit with respect to activation by protein and lipid thromboplastic agents. Prothrombin was isolated by the technique of Munro and Munro (14) from plasma of a normal rabbit and the Dicumarol plasma of *rabbit 6*. These prothrombin solutions had a Kjeldahl nitrogen value of 5 and 6 mg. per cent and thus represented a purification of the order of 250 times. Experiments on the conversion of prothrombin to thrombin by lipid and protein thromboplastic agents were carried out by determining the clotting time of 0.3 ml. of fibrinogen, 0.1 ml. of prothrombin, 0.1 ml. of one per cent CaCl₂ and 0.1 ml. of the thromboplastic agents. Similar tubes were set up using 0.1 ml. of one per cent NaCl instead of the thromboplastic agents. Since clotting did occur in these latter tubes, it is evident that an extraneous thromboplastic agent normally present in plasma

had been carried over in the preparation of either the fibrinogen or the prothrombin or both.   The clotting times in seconds of these mixtures are given in table 4.

The data of table 4 are in agreement with the results found using whole Dicumarol plasma.   The prothrombin from untreated rabbits was converted to thrombin more rapidly by the protein thromboplastic agent than by the lipid.   In the case of the prothrombin isolated from the Dicumarol-treated rabbit, the clotting time of the protein agent was longer than that of the control saline solution, while the lipid clotting time was considerably shorter than that of the control.   Thus it appears that the thromboplastic lipid is able to convert to thrombin the purified prothrombin obtained from plasma of Dicumarol-treated rabbits while the protein agent is inactive.   Furthermore, the protein agent actually inhibits clotting of these solutions by the extraneous thromboplastic agent present in the preparations.   Substantially the same results were obtained with another isolation from Dicumarol plasma.

<div align="center">DISCUSSION</div>

The fact that a lipid thromboplastic agent clots the plasma of Dicumarol-treated rabbits, whereas the normally more potent protein thromboplastic agent is ineffective, necessitates a change in concept either of thromboplastic action or of Dicumarol action.   An unlikely explanation is that there are two prothrombins in the blood, one activated by the protein thromboplastic agent and the other by the lipid thromboplastic agent.   With this hypothesis one would assume that the Dicumarol affects primarily the synthesis of the latter prothrombin.   This is thought unlikely, since adding highly purified prothrombin to Dicumarol plasma will not only make it clottable by a ·protein clotting agent but, also, reduces the somewhat prolonged clotting time of the lipid thromboplastic agent to its normal value.   This indicates that both the lipid and the protein thromboplastic agents can react with the same prothrombin.

It is believed that the results can best be explained by assuming that Dicumarol does not prevent the formation of prothrombin by the liver but changes the specificity of prothrombin formed by the liver in such a way that it is not activated by protein thromboplastic agents but is activated, though with decreased efficiency, by lipid thromboplastic agents.   Thus, consider the blood of *rabbit 6*.   Before Dicumarol, its clotting time with a protein thromboplastic agent was 57 seconds.   After Dicumarol treatment it was greater than 11,000 seconds.   From these values and the usual concept of Dicumarol action, one would expect that the plasma was practically devoid of prothrombin.   Yet, the plasma that did not clot in 11,000 seconds with a protein thromboplastic agent did clot with a lipid thromboplastic agent in 460 seconds.   Furthermore, the 'prothrombin' in the Dicumarol plasma was purified and experiments showed it to be acted on by the lipid agent but not by the protein agent.   We think that this is strong evidence for the presence of an altered prothrombin in Dicumarol plasma, especially since experiments in which the prothrombin content of normal plasma is decreased by either dilution or adsorption on aluminum hydroxide show that the protein thromboplastic agent is always more active than the lipid.

The somewhat prolonged clotting time of Dicumarol plasma with the lipid throm-

boplastic agent could indicate either that the altered prothrombin is present in a lower concentration than normal plasma prothrombin or that the efficiency of conversion of the altered prothrombin by the lipid is decreased. The possibility that both of these contribute to the prolonged clotting time also exists. While no rigorous argument could be developed from the data favoring any of these choices, it is believed more probable that the lipid agent acts on the altered prothrombin with less efficiency. The very fact that the prothrombin has a changed structure makes it unlikely that it will react with the lipid thromboplastic agent with the same reaction velocity constants as does native prothrombin.

The results further indicate that the protein thromboplastic agent actually inhibits the conversion of prothrombin to thrombin by the lipid thromboplastic agent. This behavior readily explains the results of the workers who used crude thromboplastic mixtures containing both protein and lipid thromboplastic substances. Such a mixture is the thromboplastin prepared by Quick's method (16) and used extensively by workers to determine clotting times of Dicumarol plasma. The protein thromboplastic agent in these preparations probably prevents the lipid thromboplastic agent from exerting its effects. Consequently, measurements using such thromboplastic preparations would be the same as those obtained using protein thromboplastic agents.

The clotting of recalcified Dicumarol plasma, when one per cent NaCl is used instead of a thromboplastic agent, indicates that the extraneous thromboplastic substance present in the rabbit plasma has the characteristics of the lipid thromboplastic agent. Its efficiency of action is decreased in the case of Dicumarol plasma and its action is inhibited by the protein thromboplastic agent. This view receives further support from the experiments studying the conversion to thrombin of prothrombin purified from Dicumarol plasma. It was shown that considerable extraneous thromboplastic substance is carried over in the preparation of either the prothrombin or the fibrinogen and this substance behaves in much the same fashion as the lipid thromboplastic agent.

SUMMARY

Experiments were carried out on the clotting of plasmas of untreated rabbits and rabbits treated with Dicumarol using lipid and protein thromboplastic agents. While with normal rabbit plasma the protein thromboplastic agent is more active than the lipid agent, the relationship is reversed after treatment with Dicumarol. In one case Dicumarol plasma which did not clot in three hours with the protein agent clotted within five minutes with the lipid agent.

Since reduction of prothrombin concentration by dilution or by adsorption on aluminum hydroxide does not reverse the relative thromboplastic activities of the lipid and protein agents, it is assumed that Dicumarol treatment does not prevent the synthesis of prothrombin by the liver but causes the synthesis of an altered prothrombin. This altered prothrombin is not converted to thrombin by the action of a protein thromboplastic agent but is converted by the action of a lipid agent.

It has been shown that the protein thromboplastic agent inhibits the clotting of Dicumarol plasma by the lipid thromboplastic agent. This fact is thought to ex-

plain the results of prothrombin time determinations on Dicumarol plasma using a thromboplastic agent containing both the lipid and protein agents. The thromboplastic substance normally present in rabbit plasma has characteristics of the lipid thromboplastic agent.

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EFFECT OF THYROXINE AND THIOURACIL ON THE RATE OF PHOSPHOLIPID TURNOVER IN THE LIVER OF THE RAT[1]

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CALCULATIONS based on the exchange of P^{32} from the inorganic phosphate to the phospholipids of the liver indicate that approximately 5 per cent of these phospholipids are renewed each hour in the normal adult rat (1). Very large variations of the amount of protein, carbohydrate or fat in the diet of the rat may produce alterations of the relative size of the liver and the concentration of phospholipids of the liver. The rate of phospholipid formation, however, under these circumstances was found to be essentially that of normal rats (2). A marked increase has been found in the rapidly regenerating liver after partial hepatectomy so that the amount of phospholipid formed by the remaining portion of the liver approximates that formed by the entire liver of a normal rat (3). An increased turnover rate, as indicated by an increased amount of administered P^{32} incorporated in the hepatic phospholipid, has been found after the administration of choline (4), betaine, methionine (5), cystine and cysteine (6) and a decrease has been found after administration of cholesterol (7).

The purpose of the experiments reported here was to determine the changes in the distribution of administered P^{32} in the inorganic phosphate and the phospholipids of the plasma and liver in rats after the administration of thyroxine or thiouracil. Calculations made from these data indicate that the rate of turnover of the phospholipids of the liver is increased by the administration of thyroxine and decreased by the administration of thiouracil.

EXPERIMENTAL METHODS

Adult male white rats weighing approximately 200 gm. maintained on the stock commercial diet of Friskies were fasted for 20 hours prior to the administration of P^{32}. One group of rats had previously received two daily subcutaneous injections of 0.1 mg. of thyroxine/100 gm. of body weight and a second group had received 0.1 per cent of thiouracil in the drinking water for periods of from 10 to 56 days. The basal metabolic rate of the rats receiving thyroxine was increased and sections of the thyroids, taken at the time of necropsy, showed the alterations expected after the administration of thiouracil. Twelve microcuries of P^{32} from the cyclotron at the Massachusetts Institute of Technology were injected intravenously, as dibasic sodium phosphate containing less than 0.01 mg. of phosphorus, at intervals of one-twelfth, one-fourth, one, two and four hours prior to removal of blood and liver, with the rats under pentobarbital sodium anesthesia. The inorganic phosphate and phospholipid of plasma and liver were extracted and the concentration and radioactivity of each were determined by methods previously described (1).

<div style="text-align:center">RESULTS</div>

In both the thyroxine-treated and thiouracil-treated rats the weight of the liver and its water, total lipid, phospholipid and inorganic phosphate content were well within the range for normal rats fasted for the same interval.   The plasma from the thyroxine-treated rats contained definitely increased amounts of inorganic phosphate, but normal amounts of phospholipid.   The plasma from the thiouracil-treated rats
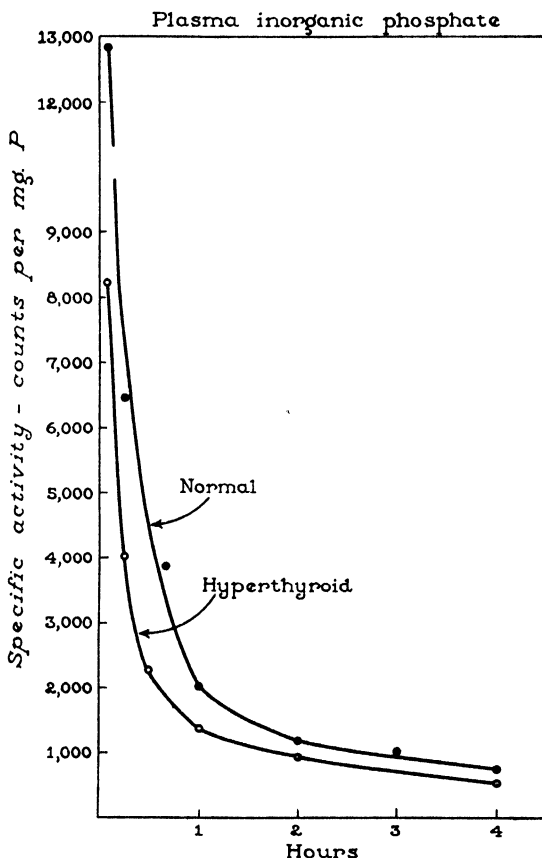

Fig. 1.   Curve of mean values found in normal and thyroxine-treated rats for specific activity of plasma inorganic phosphate after a single injection of phosphate P^{32}.

contained slightly less than normal amounts of inorganic phosphate and slightly more than normal amounts of phospholipid.

Because of the greater amount of inorganic phosphate in the plasma of the hyperthyroid rats, the injection of the same amount of P^{32} produced definitely lower average specific activity of the plasma inorganic phosphate than in normal rats (fig. 1), which in turn produced lower specific activity of the inorganic phosphate of the liver.   The opposite was true for the thiouracil-treated rats, in which the specific activity of the inorganic phosphate of the plasma and of the liver was higher than in normal rats.

The specific activity of the phospholipids of the livers of the thyroxine-treated rats was almost identical with that of normal rats at each of the time intervals studied. The radioactivity of the phospholipid was acquired from the inorganic phosphate, which had less activity than that of the normal rats, so that more phospholipid must have been formed in the hyperthyroid rats. The relative activity, which has often been used as an indication of the rate of turnover, was 47 per cent in the hyperthyroid rats as compared with 31 per cent in the normal rats and with 22 per cent in the hypothyroid rats four hours after the administration of P³². The specific activity of the phospholipids of the liver of the hypothyroid rats was definitely less than that of normal rats taken at the same time interval after administratio of P³².
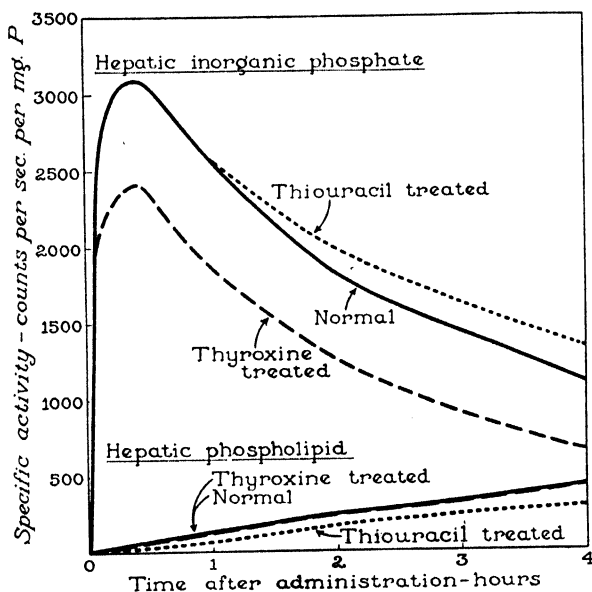
Fig. 2. CURVE OF MEAN VALUES of specific activities of hepatic inorganic phosphate and of hepatic phospholipid found after a single intravenous injection of phosphate P³².

For the calculation of the rate of turnover of the phospholipids of the liver we have used the method of Zilversmit, Entenman and Fishler (8), who demonstrated that the turnover time of substance B, which is formed from the precursor A, is the quotient of the difference of the areas of the specific activity time curves of the two substances divided by the change of specific activity of B during the time interval. We have used the specific activity of the liver inorganic phosphate as that of the precursor of the liver phospholipid. The proportion of the liver phospholipids, which is renewed each hour (R) is the quotient of the change of specific activity of the phospholipid per hour ($d\ pl$) divided by the difference of the mean specific activity of the liver inorganic phosphate ($m\ i$) and the mean specific activity of the liver phospholipid ($m\ pl$). $R = (d\ pl)/m\ i\text{-}m\ pl$. The mean specific activity for the inorganic phosphate and phospholipid phosphorus, which is required for the calculation of R, was obtained by evaluating the respective areas under the time curves (fig. 2) and

relating this to the time interval considered. The chief numbers of interest are summarized in table 1. The corresponding activity-time curves were obtained by graphic smoothing of the observed specific activities in order to depict the course of changes of these values. The numbers pertinent to the calculation of R are to be found in rows 12 through 16 of the table. The rate of change of specific activity of hepatic phospholipid per hour (row 15) is obtained by dividing the specific activity at time t (row 10) by the time (row 1). The mean specific activities of the hepatic inorganic P and phospholipid P (rows 12 and 13) were obtained from the area under the time activity curves and their differences are given in row 14. The value for R

TABLE 1

	THYROXINE-TREATED RATS				THIOURACIL-TREATED RATS		
1. Hrs. after administration, t	0.5	1	2	4	1	2	4
2. Rats	6	10	12	14	6	23	38
3. Body weight, gm.	217 ± 6	177 ± 5	180 ± 3	176 ± 2	204 ± 4	223 ± 4	210 ± 2
4. Liver wt., % of body wt.	3.4 ± 0.1	4.5 ± 0.3	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.6 ± 0.2	4.0 ± 0.1
Mg. P/100 gm. (items 5 to 7)							
5. Plasma inorg. P	7.3 ± 0.3	9.2 ± 0.4	10.2 ± 0.4	9.4 ± 0.3	6.7 ± 0.1	5.9 ± 0.2	6.0 ± 0.2
6. Liver inorg. P	27.6 ± 0.6	30.4 ± 0.9	28.1 ± 0.8	31.6 ± 1.0	32.2 ± 0.3	29.8 ± 0.5	30.4 ± 0.5
7. Liver lipid P	129 ± 1	121 ± 4	118 ± 1	125 ± 2	126 ± 3	128 ± 2	131 ± 2
Specific activity at time t (items 8 to 10)							
8. Plasma Inorg. P	2,227 ± 120	1,372 ± 38	929 ± 66	509 ± 32	2,665 ± 93	1,440 ± 55	843 ± 23
Lipid P		23 ± 3	164 ± 14	283 ± 7	23 ± 3	51 ± 5	185 ± 10
9. Liver inorg. P	2,364 ± 162	1,879 ± 61	1,267 ± 72	681 ± 46	2,585 ± 93	1,985 ± 59	1,339 ± 30
10. Liver lipid P	64 ± 9	110 ± 11	257 ± 14	320 ± 21	72 ± 3	179 ± 14	284 ± 9
11. Relative activity, liver lipid P: inorg. P	0.028	0.058	0.208	0.47	0.028	0.088	0.223
Mean sp. act. to time t (items 12 to 14)							
12. Liver inorg. P	1,961	2,038	1,800	1,375	2,641	2,463	2,062
13. Liver lipid P	30	62	123	206	36	80	154
14. Difference	1,931	1,976	1,677	1,169	2,605	2,383	1,908
15. Change of specific activity of lipid P/hr.	128	110	128	80	72	89	71
16. R	0.066	0.056	0.076	0.069	0.028	0.037	0.037
17. New phospholipid P in mg/hr/100 gm. of rat	0.29	0.30	0.33	0.32	0.13	0.17	0.19

is given in row 16. The amount of phospholipid P newly formed each hour calculated for each 100 gm. of rat ($R \times$ mg. phospholipid P/100 gm. liver · liver weight/ body weight) is given in row 17. This is an average of 0.31 mg. for the 42 thyroxine-treated rats and 0.18 mg. average for 67 thiouracil-treated rats, which differs by $+29$ per cent and -25 per cent respectively from the average of 0.24 found for 45 normal adult rats (1) at similar periods after administration of P^{32}.

We have made similar calculations from the data, obtained one hour after administration of P^{32}, from 8 young normal rats weighing 75 gm. each. These showed a turnover rate of liver phospholipids of 0.054 and because the liver is relatively larger in the young rats than in older rats 0.33 mg. of phospholipids was being formed for each 100 gm. of rat each hour. Twelve other young rats weighing 123

gm. taken four hours after administration of P³² gave data from which 0.058 for R and 0.33 mg. for the amount of new phospholipid P formed each hour/100 gm. of rat were calculated. For these two groups of young animals the rate of phospholipid turnover in the liver was approximately that of normal adult rat liver, but was greater on the basis of total body weight.

On the assumption that the new phospholipid entering the plasma has the same specific activity as the average specific activity of the hepatic phospholipids, we have attempted to calculate the rate of turnover of the plasma phospholipids. The specific activity of the liver phospholipid was considered to be that of the precursor of the plasma phospholipids and calculations were made from the smoothed curves of the specific activity-time relations. There was considerable variation in the data from individual rats at the same time periods. The turnover rate, that is, the proportion of phospholipids replaced in the plasma each hour, was calculated as 0.79 from average data from 38 adult normal rats taken one, two or four hours after administration of P³². Similar data from 36 thyroxine-treated rats gave 0.97 or an increase of 23 per cent over the rate found for normal rats. Sixty-seven adult thiouracil-treated rats gave 0.63 or a decrease of 20 per cent from that of the normal rats.

<div align="center">COMMENT</div>

That the thyroid hormone may cause changes in the distribution of such inorganic constituents of the body as calcium and phosphorus was shown by Aub *et al.* (9), when they found that the hormone causes a marked increase of the excretion of both phosphorus and calcium. Aoike (10) found an increased concentration of inorganic phosphate in the blood of the rabbit associated with the increased excretion of phosphorus. Logan, Christensen and Kirklin (11) found an increased excretion of calcium without significant changes in phosphorus in the dog. Robertson (12) found decreased concentration of both serum calcium and phosphorus in active untreated thyrotoxicosis in human beings. In our hyperthyroid rats there was an increased concentration of inorganic phosphate in the plasma. A similar finding has been reported by Greenberg, Fraenkel-Conrat and Glendening (13). The more rapid disappearance of injected radioactive inorganic phosphate in the hyperthyroid rats than in normal ones is in part explained by the greater dilution which occurs in plasma containing an increased concentration of inorganic phosphate. Also the increased circulation time and increased rate of excretion of phosphorus found in hyperthyroid animals are undoubtedly contributory factors. Of particular interest in this connection is the recent observation of Greenberg, Fraenkel-Conrat and Glendening (13), of a large increase in the rate of penetration of inorganic phosphate into the muscle of hyperthyroid animals.

There is definitely a relationship between the rate of turnover of hepatic phospholipid and the rate of basal metabolism, since both rates can be elevated or depressed by the administration of thyroxine or thiouracil. This may be related to the oxygen consumption in the liver, since Taurog, Chaikoff and Perlman (14) have shown that aerobic conditions are essential for the synthesis of phospholipid from radioactive inorganic phosphate in surviving liver slices.

The validity of the assumption, made for the purpose of calculating the turn-

over rate of hepatic phospholipids, that the specific activity of the hepatic inorganic phosphates is similar to that of the immediate precursor of the hepatic phospholipids is further enhanced by these studies. Previous studies indicated that this method of calculation gave constant results when calculated from entirely different curves obtained after single injections of P^{32} or after continuous administration of P^{32} and also from 10 different intervals on those curves. The rate of phospholipid turnover in the liver of thyroxine-treated animals was found to be greater than normal when calculated at four different periods after administration of P^{32} and to be reduced consistently in thiouracil-treated animals for the three different periods used.

The specific activity of the liver phospholipids of the thyroxine-treated animals was almost identical with that of untreated animals at similar time periods after the administration of the same amount of P^{32}-labeled inorganic phosphate. If the rate of turnover of phospholipid were estimated from the percentage of the administered P^{32} which appeared in the phospholipids, no change from normal would be noted. It is obvious that any condition, as in this case an elevation of the inorganic phosphate content of the plasma, which alters the specific activity of the precursor would correspondingly alter the specific activity of any compound formed from that precursor. In the thiouracil-treated animals the specific activity of both the inorganic phosphate and the phospholipid differed from that found in normal animals; less P^{32} was incorporated in the phospholipids of the liver from inorganic phosphate of higher specific activity. The rate of turnover of phospholipids was therefore less than it would be judged to be from the percentage of the administered P^{32} which appeared in the phospholipids.

The calculation of the turnover rate of plasma phospholipids, based on the assumption that the average specific activity of the phospholipids of the liver is the same as that of the newly formed phospholipids entering the plasma, is of course open to question. The specific activity of the newly formed phospholipids of the blood may have been very different from that of the average of the liver phospholipids. However, the calculated rate of turnover did not vary greatly at different periods of these experiments when the changing values would have indicated changing rates if any appreciable disparity existed. In view of this consistency, the finding of an increased rate after administration of thyroxine and a decreased rate after administration of thiouracil may provisionally be accepted. The calculated rate of phospholipid turnover in the normal rat of 0.79 should be further checked by calculations made after continuous injection of P^{32} and also by the rate of disappearance of labeled phospholipid from the plasma (15).

SUMMARY

Data concerning the concentration and specific activity of the liver and plasma in organic phosphates and phospholipids were obtained from adult rats at intervals after the injection of dibasic sodium phosphate P^{32}. Because their specified activities have a constant relationship over a wide variety of experimental conditions, the specific activity of the liver inorganic phosphates appears to be identical with that of the immediate phosphate precursor of the phospholipids of the liver. Calculations based on this assumption indicate that the rate of phospholipid turnover in the liver

is increased in rats which have received thyroxine and decreased in rats which have been treated with thiouracil. The rate of turnover of the phospholipids of the plasma also appears to be greater than normal in thyroxine-treated rats and less than normal in those receiving thiouracil. The rate of phospholipid turnover in the liver of young rats is essentially the same as that of mature rats but, since the relative size of the liver is greater in young rats, more phospholipid is formed in proportion to body weight than in the adult.

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EFFECT OF SUPRATHRESHOLD CHANGES IN BRIGHTNESS ON FORM PERCEPTION

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THE detrimental effect of diminishing brightness on seeing during the post-sunset hours has been generally recognized although not subjected to systematic study. A subjective vagueness occurs during dusk although the general level of illumination is suprathreshold. Investigation of dark adaptation (1) has dealt almost exclusively with the measurement of the absolute threshold for the perception of light, the suprathreshold brightness range not having been studied. The investigation here reported was designed to test the effect of suprathreshold brightness changes on form perception.

Variables of experimental procedure were introduced in an effort to reproduce the essential conditions under which poor dusk seeing occurs. The approach was based on the supposition that the anticipated changes, although occurring at suprathreshold levels, were of primarily adaptive nature. Since adaptive processes have been recognized to be impaired after exposure to intense brightnesses (2, 3), a regimen of preadaptation to bright light was introduced. To simulate field conditions, where high contrasts are seldom encountered, a set of low contrast test objects was used in certain of the experiments. The fact that poor dusk seeing is accompanied by poor confidence in having seen correctly suggested the inclusion of a measure of the subject's appraisal of his own seeing ability for comparison with his measured physiological ability.

APPARATUS

Perimeter and test objects. Tests were made in a white cloth testing booth on a 25-cm. perimeter, on which the test objects were illuminated by a 60-watt Mazda daylight bulb. A voltage regulator (Sorensen and Co. Model 150) designed to keep the output voltage at 115 with regulation accuracy of 0.5 per cent in a recovery time of six cycles ($\frac{1}{10}$ sec.) on an input voltage of 95 to 125 volts was operated in the power line in all experiments. The brightness of the test object was regulated by a rheostate and measured with a Macbeth Illuminometer at arbitrarily chosen resistances. During experimentation the brightness changes were controlled by setting the rheostat at the measured resistances. An arrangement of white hand-operated blind, test object with surrounding white field 74 mm. square, and white background reflector presented to the subject's visual field a 60° circular patch of uniform brightness, which did not measurably change in brightness as the test object was alternately presented and obscured. When the blind was turned for presentation the test object was revealed in the center of the 60° field. The test objects were Landolt circles. They were photo-

graphically printed on white background. Notation of their sizes was indicated by the mm. measure of the break. The contrast of the test object against the white background is conventionally expressed as a percentage based on relative brightnesses. The test object could be turned about its own center so that the break in the circle pointed in one of four directions; up, down, right or left.

Adaptation booth. A booth 4 feet X 4 feet X 8 feet was constructed for the purpose of light adapting the subjects. The inside walls were coated with magnesium carbonate. The booth was illuminated by 16 40-watt daylight fluorescent bulbs. The brightness of the front and side walls at sitting height was approximately +2.7 log millilamberts, at standing height +2.8 log millilamberts. During light adaptation the subjects sat in the booth, the sitting height brightness level being used in this report.

TECHNIQUE

. The test objects were presented to the subjects for positional identification, the subjects' responses being an indication of the position of the break in the circle. When the subject answered the blind was returned to the obscuring position and the test object repositioned for representation. Presentations were made in sequences of 20 each, there being in each sequence five presentations of each of the 4 possible positions. The order of positional presentation was unknown to the subject and was determined by chance, the operator working from a prepared sheet. The subjects responded promptly. Presentations were made unhurriedly at the rate of one sequence of 20 in one minute and 15 seconds. The actual time of each stimulus exposure was somewhat less than 2 seconds. When positional identification was difficult by reason of small test object size or low brightness there was a tendency for the subject's responses to lag, but forced guesses were the requirement. Even under these conditions the prescribed speed of presentation was maintained without difficulty. Special operations such as time notation etc. between consecutive sequences were found to require an interval of about 15 seconds which was adopted for routine use. When change of brightness was required the rheostat was manipulated immediately before the first presentation in the new sequence. With the test object already positioned the brightness was changed and the operator's hand then turned the blind to make the first presentation. The manipulation of the rheostat required about one second, the first presentation following in less than one second. When the experiment required a change of test object size before continuing, a longer interval than 15 seconds was found expedient. A half minute was usually allotted but this varied somewhat.

The subject was required to state his confidence in having given the positional identification correctly. After each group of 4 presentations (every 15 sec.) the subject was asked, "How many?" His answer, from 0 to 4, covered the foregoing 4 presentations and gave the number of responses which he was certain he had gotten right.

A single sequence, comprising 20 presentations, was adopted as the unit of scoring. The responses to stimulus presentation are a measure of physiological efficiency and are referred to in this report as the 'performance'. The number of correct answers are totaled for each sequence and are converted to a percentage by multiplying by five. In this form the percentile performance scores are not a direct measure of the subject's ability since there is 1 chance in 4 that any response might have been correct by chance. Therefore the percentile performance scores have been corrected as

follows; $P_{(cor)} = (P_{(uncor)} - 25)/75 \times 100$ where $P_{(cor)}$ represents the corrected performance score and $P_{(uncor)}$ the uncorrected performance score. The rationale of this correction is illustrated by the following example. Suppose that a subject's true frequency of seeing is known to be 60 per cent. Then the remaining 40 per cent of presentations will have one answer in four correct by chance or 10 per cent of the total. The uncorrected performance score will be $60 + 10 = 70$ per cent, which corrects by the above formula to the true value. The responses covering certainty of having seen correctly are a measure of the subject's conscious sensory experience and are referred to in this report as the 'confidence.' The confidence scores have been similarly converted to percentages for each sequence, but they need no correction because they possess no element of chance. After the performance scores have been corrected they are directly comparable to the confidence scores. In this report all scores are reported as percentage frequency of seeing, usually for a sequence of 20 presentations .

<div align="center">PROCEDURE</div>

Areas far removed from the line of central vision are best suited to investigations involving brightness changes for the significant reason that photopic and scotopic acuity are nearly the same 30° or more peripherally (4, 5). The writer's previous measures of photopic and scotopic form acuity at 30° and 60° peripherally have shown them to be related as 7 is to 5, the former being the stronger (6). Therefore, in the whole range of possible brightnesses the form acuity ought to vary only slightly if at all for the different levels, providing the eye has had time to adapt to the new brightness. This circumstance should facilitate the interpretation of any changes observed, since it has eliminated the possibility of there being greatly different acuities for the different brightnesses used.

Preliminary experiments were performed during the development of the technique. Since the number of subjects was small these experiments are not reported in detail. The results indicated that a one-half log unit sudden diminution of brightness does not measurably depress peripheral form perception at 30° and 45° peripherally at brightnesses from $+0.8$ to -1.6 log mL. At lesser brightnesses both confidence and performance are depressed although the absolute threshold is not passed. Differential threshold curves based on different-sized test objects are sigmoid and asymptote at 0 per cent and 100 per cent after proper correction of the performance scores.

It was considered to be of interest to demonstrate changes in form perception following suprathreshold brightness diminution at as high a level as practical to emphasize the occurrence of the phenomenon at the higher levels of brightness. A series of 12 experiments, described in table 1, was performed on a group of 11 subjects. Among these experiments are two groups of three experiments each, the first group being performed with low contrast (27%) test objects 45° peripherally (*expts. 1, 2, 9*) and the second with high contrast (97%) test objects 30° peripherally (*expts. 3, 4, 10*). In these experiments the effect of a suprathreshold brightness diminution of 2.8 log units was tested with identical technique under the three following conditions of adaptation, *a*) no preadaptation, *b*) preadaptation to $+2.7$ log mL. for $1\frac{3}{4}$ hours and *c*) preadaptation to $+2.7$ log mL. for $1\frac{3}{4}$ hours with pupils dilated by 5 per cent euphthalmine 15 minutes before the preadaptation began. The largest test object

was used first, the subjects receiving one series of 20 presentations at $+1.2$ log mL., then a second sequence at -1.6 log mL. and finally a third sequence at the original brightness. Then after an interval of about one-half minute the procedure was repeated with a smaller test object and finally with the smallest.

Experiments 5 and *6* with 27 per cent contrast and *7* and *8* with 97 per cent contrast provided differential threshold curves for peripheral form perception in the fully adapted eye at the two levels of brightness used in the experiments above, $+1.2$ and -1.6 log mL. At the higher level no preadaptation was considered necessary, but in *experiments 6* and *8* which were conducted at -1.6 mL. the subjects wore Navy dark adaptor goggles for one-half hour previous to the test and were not exposed to greater brightnesses in the interim. Test objects were presented for one sequence each in

TABLE I. DATA FOR SERIES OF EXPERIMENTS

EXPT. NO.	ANGLE DEV.	TEST OBJECT SIZE	TEST OBJECT CONTRAST	TEST OBJECT BRIGHTNESS IN LOG mL	PREPARATION OF SUBJECTS
1	45°	4, 3, 2	27%	+1.2 and −1.6	None
2	45°	4, 3, 2	27%	+1.2 and −1.6	1¾ hrs. at +2.7 log mL.
3	30°	2, 1, ½	97%	+1.2 and −1.6	None
4	30°	2, 1, ½	97%	+1.2 and −1.6	1¾ hrs. at +2.7 log mL.
5	45°	6, 5, 4 3, 2	27%	+1.2	None
6	45°	6, 5, 4, 3, 2	27%	−1.6	Dark adapted ½ hr. with Navy goggles
7	30°	2½, 2, 1½, 1, ½, ¼	97%	+1.2	None
8	30°	2½, 2, 1½, 1, ½, ¼	97%	−1.6	Dark adapted ½ hr. with Navy goggles
9	45°	4, 3, 2	27%	+1.2 and −1.6	Pupils dilated: 1¼ hrs. at +2.7 log mL.
10	30°	2, 1, ½	97%	+1.2 and −1.6	Pupils dilated: 1¼ hrs. at +2.7 log mL.
11	30°	2	97%	+1.2 and −1.6	None
12	30°	2	97%	+1.2 and −1.6	Pupils dilated: 1¼ hrs. at +2.7 log mL.

order of descending size. The test object contrast and angular deviations were the same as in the remainder of the experiments.

Experiments 11 and *12*, utilizing the same brightness diminution as other experiments, were designed to measure the duration of the observed perceptual changes. A single high contrast (97%) test object, size 2, was used 30° peripherally. The subject received one sequence at $+1.2$ log mL. followed by 10 sequences at -1.6 log mL., after which the original brightness was restored for one sequence. The subjects were not preadapted in *experiment 11*, but in *experiment 12* were light adapted at $+2.7$ log mL. for 1¾ hours with dilated pupils.

The group of 11 subjects who took the above 12 tests, with one exception (the writer), were students at The Johns Hopkins University. No attempt was made to select them for any reason other than convenience of schedule. Each subject received an examination by a practicing ophthalmologist. No ocular pathology other than mild routine optical defects was discovered.

<div align="center">RESULTS</div>

Figure 1 illustrates the effect of altered brightness on the form perception of low contrast (27%) test objects presented 45° peripherally on the horizontal meridian. The solid cross bars represent the mean corrected performance scores in 11 subjects
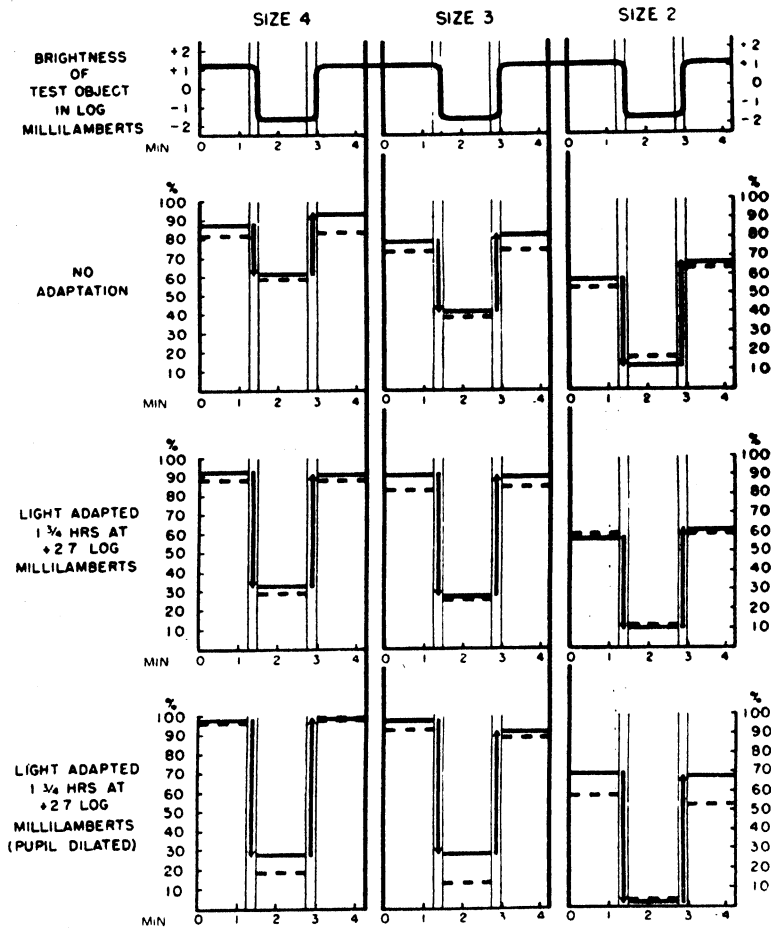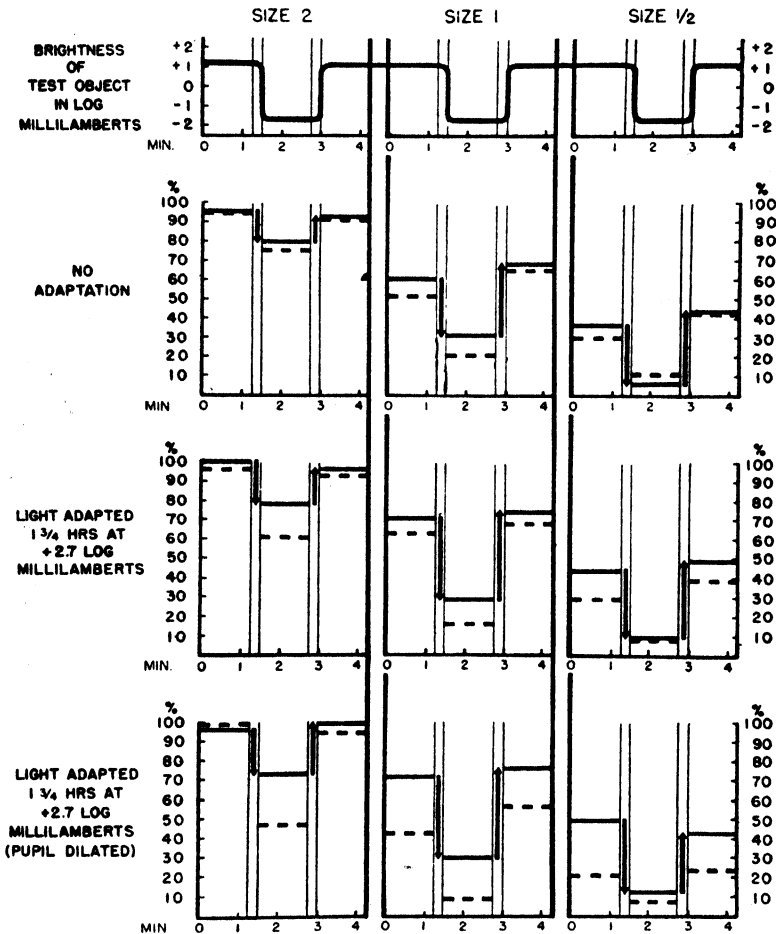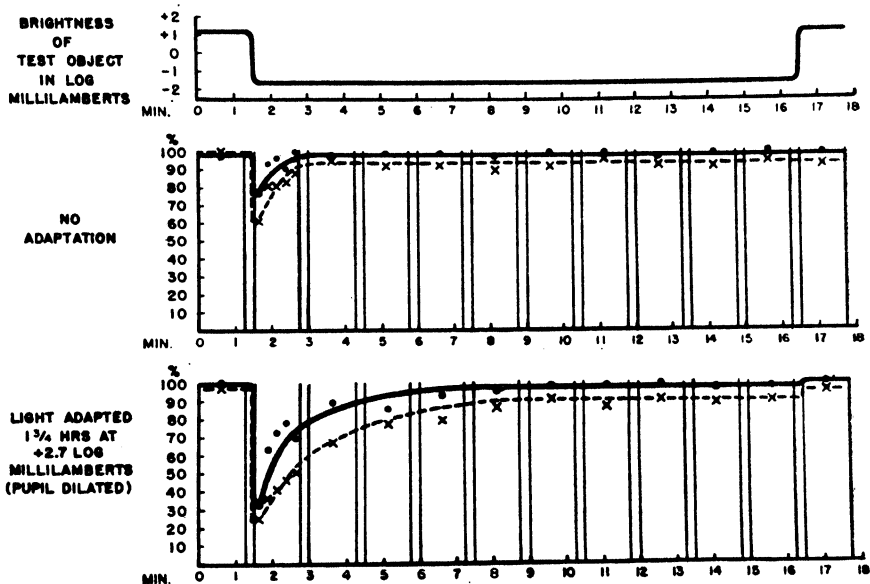

Fig. 1. EFFECT OF CHANGING BRIGHTNESS on peripheral form perception. Average scores of 11 subjects are expressed as the percentile frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec. The tests were made at 45° on the horizontal meridian of the right eye. Test objects were Landolt circles of 27% contrast with breaks of 4 mm., 3 mm. and 2 mm. Testing intervals between test object sizes were about ⅓ min. each. Solid cross bars represent the actual frequency of seeing; dashed cross bars, the subject's certainty of having given correct responses. Experiments began ⅓ min. after the end of the light adaptation period. Order of testing progresses from left to right and top to bottom.

for each sequence. The dashed cross bars similarly represent the confidence. The performance is depressed by brightness diminution from +1.2 to −1.6 log mL. for all test object sizes and adaptive conditions used. When the original brightness was restored the scores returned to approximately the original level. The loss was greater

when the subjects had been previously light-adapted with normal pupil and still greater after light adaptation with pupils dilated. The statistical significance of the difference between the scores at $+1.2$ log mL. and those at -1.6 log mL. was checked
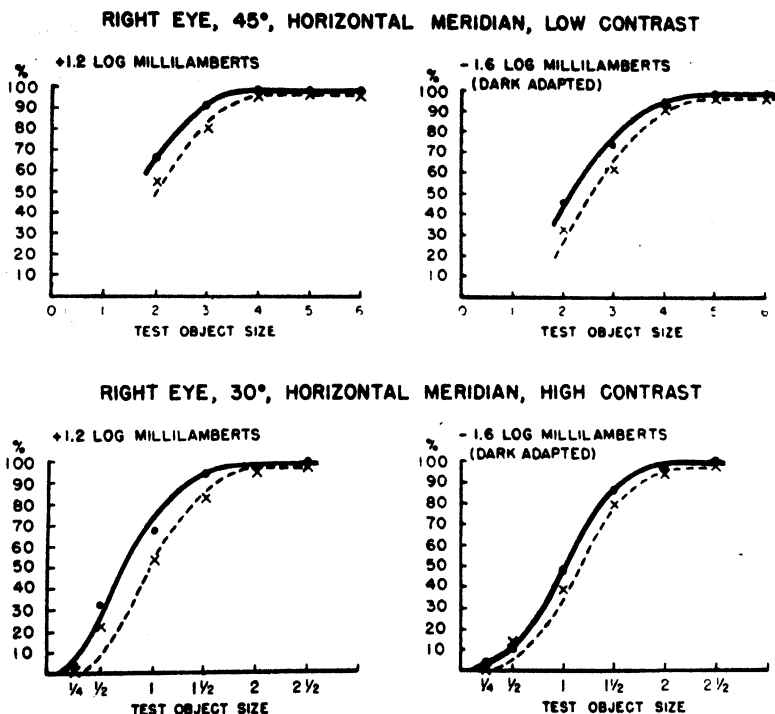

Fig. 2. EFFECT OF CHANGING BRIGHTNESS on peripheral form perception. Average scores of 11 subjects are expressed as the percentile frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec. The tests were made at 30° on the horizontal meridian of the right eye. Test objects were Landolt circles of 97% contrast with breaks of 2 mm., 1 mm. and ½ mm. Testing intervals between test object sizes were about ½ min. each. Solid cross bars represent the actual frequency of seeing; dashed cross bars, the subject's certainty of having given correct responses. Experiments began ½ min. after the end of the light adaptation period. Order of testing progresses from left to right and top to bottom.

by small sample theory formulae (7, p. 59). The first group represented in figure 1 (size 4, no adaptation) has means that are significant at the 2 per cent level of confidence. All of the rest are significant at the one per cent level of confidence. The scores were analyzed to determine whether or not the performance loss in eyes light-

adapted with dilated pupils was significantly greater than the loss sustained by the same subjects when unadapted.   In other words, did the light adaptation produce a significantly greater effect?   The increase in the depression of the form perception was significant at the one per cent level of confidence with *test objects 4* and *3* and at the 2 per cent level for *size 2*.   The mean confidence scores are somewhat less than the mean performance scores and do not show any independent trend.

Figure 2 illustrates the effect of changing brightness on the form perception of high contrast (97%) test objects presented 30° peripherally on the horizontal merid-an.   Here again, losses have occurred from diminished brightness in all cases.   The

Fig. 3.   DURATION OF EFFECT OF CHANGING BRIGHTNESS on peripheral form perception.   Average scores of 11 subjects are expressed as the frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec.   The tests were made at 30° on the horizontal meridian of the right eye.   The test object was a Landolt circle of 97% contrast with a break of 2 mm.   Dots and solid curves represent the actual seeing; crosses and dashed curves, the subject's certainty of having given correct responses.   The scores of the sequence following brightness diminution have been broken down into 15 sec. intervals.   The experiment following light adaptation began ½ min. after the end of the light adaptation period.   The curves are drawn by inspection.

differences between the performance scores at +1.2 log mL. and −1.6 log mL. are all significant at the one per cent level of confidence, most of them greatly exceeding the minimal difference required.   The results differ from those obtained with low con-trast (27%) test objects in that the losses observed after light adaptation, although greater, are not as marked.   Statistical analysis of the increase in loss after light adaptation with dilated pupil over that observed in unadapted eyes revealed it to be not significant.   The confidence scores show a tendency to separate from the perform-ance scores in the later experiments.

Figure 3 presents the mean scores of 11 subjects in *experiments 11* and *12* which

measured the duration of the effect of diminished brightness on form perception. The second sequence, that immediately following the brightness diminution, is broken down into five 15-second intervals because of the rapid changes taking place.   There was little loss and rapid recovery of both performance and confidence when the subjects had received no adaptation.   When the subjects had been light-adapted with dilated pupils both performance and confidence losses were measurably greater and

RIGHT EYE, 45°, HORIZONTAL MERIDIAN, LOW CONTRAST

RIGHT EYE, 30°, HORIZONTAL MERIDIAN, HIGH CONTRAST

Fig. 4. DIFFERENTIAL THRESHOLD CURVES for peripheral form perception.   Average scores of 11 subjects are expressed as the percentile frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec.   Test objects were Landolt circles, the size of which is indicated by the mm. measure of the break.   Dots and solid curves represent the actual frequency of seeing; the crosses and dashed curves, the subject's certainty of having given correct responses.   The curves are drawn by inspection.

the recovery slower.   The confidence seems to lag somewhat, a slight residual effect being noticeable as long as the low brightness was continued.

Figure 4 presents differential threshold curves for peripheral form perception in the same group of 11 subjects.   The differential threshold is quite wide, extending from approximately ¼ to 2 with the high contrast test objects, a fact which facilitates the transference of frequency of seeing losses into acuity measures as discussed in a later section of this paper.   The confidence scores occupy a position just below the performance curves, which is interpreted as the proper relative value wherever frequency of seeing is not perfect.

DISCUSSION

Statistically significant losses in form perception have been observed in response to suprathreshold diminution of brightness under all of the experimental conditions that have been systematically investigated. The phenomenon has been observed in all subjects, without individual exception.

Certain differences between the results at 45° with low contrast (27%) test objects and those at 30° with high contrast (97%) test objects are evident. The former show losses in response to diminished brightness which increase significantly as the subject's exposure to bright light increases. A lesser increase occurred with the high contrast (97%) test objects but was not statistically significant. Although the reason for this difference is not clear, it is interesting to note that the low contrast test objects represent a closer approach to field conditions and probably afford a better reflection of practical function.

The differential threshold curves in figure 4 give the performance and confidence scores to be expected for the experimental conditions represented in figures 1, 2 and 3, except for the state of adaptation. In figure 4 the eyes have been adapted to the lower brightness. The level to which any performance or confidence ought to drop because of lesser efficiency at a dimmer illumination is indicated by these curves. The means in figures 1 and 2 and the readings on the differential threshold curves of figure 4 are almost the same for the higher brightness for sizes where the frequency of seeing is high. The frequency of seeing for the same test object size at the lower brightness in figure 4 is nearly the same, indicating a similar anticipated performance at the lower brightness. In spite of this there is a marked drop in frequency of seeing upon diminished brightness when the eye is not adapted to the lower brightness. For example, in figure 1, size 4, after adaptation at +2.7 log mL. for $1\frac{3}{4}$ hours with pupils dilated the performance loss of from 98 per cent to 30 per cent greatly exceeds that indicated by the differential threshold curves. On the latter the loss does not exceed 5 per cent. The performance losses exceed the anticipated losses in all cases and suggest a temporary impairment, likely of adaptive nature.

The above suspicion is largely confirmed by the experiments reported in figure 3, which measured the duration of the perceptual loss and graphed its recovery to the anticipated value. When no adaptation preceded the experiment the loss was small and the recovery rapid, being complete in about $1\frac{1}{2}$ minutes. The same technique following light adaptation with dilated pupils resulted in a greater loss which took about four times as long to recover. These curves clearly indicate the adaptive nature of the changes. In both experiments represented in figure 3 the frequency of seeing returns almost to the level for +1.2 log mL. while the brightness is still at −1.6 log mL., a circumstance predicted by the differential threshold curves of figure 4.

An estimate of changes in acuity can be made from data presented as frequency of seeing. When brightness is diminished the frequency of seeing falls to a level characteristic of a smaller test object. By assuming that the test object size indicated by the lowered frequency of seeing is to the test object size actually used as the latter is to the size required for comparable performance under the new conditions, a measure of the loss in terms of test object size can be calculated. The data in figure

3 for the first 15 seconds at −1.6 log mL. after light adaptation with pupils dilated will serve as an example. At the higher brightness frequency of seeing was 98.8 per cent, only to drop to 30 per cent upon diminished brightness for the 15-second interval mentioned above. By reference to figure 4 it will be seen that 30 per cent frequency of seeing is normally given by size ½ (approx.). Then, ½ is to 2 as 2 is to x; size 8 would be required for the original frequency of seeing during the first 15 seconds of the lower brightness. Since the stated test object size is the linear measure of the break in the circle, the areas of the two test objects are related as 1 is to 16. It then follows that, other conditions being the same, a retinal area 16 times the size of that originally stimulated would be required for responses of equal efficiency. The recovery process equates the sizes in about six minutes, when the acuity has reached the fully adapted level. An effect of comparable magnitude but probably of longer duration is evident with low contrast (27%) test objects of sizes 4 and 3. In figure 1 after light adaptation both with and without dilated pupils the frequencies of seeing losses were comparable. These scores cover the mean performance during the first $1\frac{1}{4}$ minutes in the low brightness as compared to the first 15 seconds in the cited example. This method of deriving relative acuity from frequency of seeing, although crude with the present technique and data, serves to indicate the magnitude of the changes in terms convertible into accepted expressions of visual acuity.

The brightnesses used were purposely chosen at levels above those customarily associated with adaptive phenomena. The lower brightness, −1.6 log mL., is suprathreshold for both rods and cones according to the data of Hecht (1) and Winsor and Clark (2) for the unadapted eye. This was confirmed subjectively among the present group of subjects and held true even after the light adaptation. Nevertheless the results are clearly of an adaptive nature. They closely resemble the gradual development of form acuity during the process of dark adaptation previously reported by the writer (8) for lower brightnesses. In this previous study unadapted subjects were suddenly deprived of light except for that from the test object illuminated at a low scotopic level. The time required for them to perceive the light on the test object was recorded and represented attainment of the absolute threshold for that brightness. Then their peripheral form acuity was measured by a limiting method requiring 80 to 100 per cent frequency of seeing. The form acuity was found to develop gradually to its maximal level in from 2 to 17 minutes after light was perceived. The responses of the present experiments probably represent an expression of the same phenomenon except that the brightnesses used are higher and never cross the absolute threshold. The maintainance of brightness within suprathreshold levels does not prevent the deterioration of form perception.

Interpretation of the results reported here should not be restricted to the retinal areas actually tested. Comparable effects should be expected to occur in all portions of the retina and should be anticipated in terms of the adaptive characteristics of the area in question and the adaptive state of the individual. No data can be offered at present concerning the upper limit of brightness at which significant deterioration of form perception can be produced, but it is probable that it occurs at higher brightnesses than those at which it has already been detected. The cumulative effect of regular exposure to sunlight already reported by Hecht (3) and the greater effect

after preadaptation to bright light reported here encourage such a view.   The brightness of the preadapting light used in the present experiments is scarcely an approach either in brightness or duration to that experienced by individuals living an outdoor life in sunny weather.   In such individuals in whom the absolute threshold has been raised with concomitant sluggish adaptation, the diminishing brightness of the post-sunset hours may well approach the absolute threshold closely enough to produce significant deterioration of form perception while considerable subjective brightness still persists.   The losses are primarily adaptive ones which can and doubtless do occur at levels of brightness higher than those currently associated with adaptive phenomena.

SUMMARY

The effect of sudden suprathreshold diminution of brightness from $+1.2$ log mL. to -1.6 log mL. on peripheral form perception was measured in 11 subjects.   The above brightness diminution causes a loss in frequency of seeing which is significant at the one per cent level of confidence with both high contrast (97%) and low contrast (27%) test objects.   The loss due to diminished brightness is significantly greater after the eyes have been preadapted to a brightness of $+2.7$ log mL. with dilated pupil when low contrast (27%) test objects are used.   When high contrast (97%) test objects are used the loss is greater after preadaptation as above but not significantly so.

The duration as well as the extent of the loss is increased by preadaptation to $+2.7$ log mL. with pupil dilated.   The subject's confidence in having seen correctly under these conditions is somewhat less than the actual seeing ability but maintains a fairly constant relation to it.   The changes observed are of a primarily adaptive nature and are not traceable to different acuities for different brightnesses.

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STUDY OF THE EXCHANGE OF OXYGEN AND CARBON DIOXIDE IN THE SUPRAGLOTTIC PORTION OF THE RESPIRATORY DEAD SPACE

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THE question as to whether an exchange of oxygen and carbon dioxide occurs across the mucous membranes of the respiratory dead space has concerned physiologists for many years. Henderson believed that carbon dioxide diffuses across the mucous membrane of the bronchial tree because he noted that the ratio of carbon dioxide output to oxygen consumption (*RQ*) in expired air is greater than in alveolar air (1). Subsequently, it was demonstrated by gas equilibration studies carried out over several minutes that carbon dioxide and oxygen can diffuse across the mucous membrane of the oral portion of the respiratory dead space (2).

Haldane and Priestley (3) and Krogh and Lindhard (4) did not believe that gas transfer occurred in the respiratory dead space. Haldane and Priestley postulated that the difference between the respiratory quotient of expired and alveolar air is due to unequal ventilation of the alveolar ducts and atria of the lungs and to a greater rate of diffusion of carbon dioxide than oxygen through tissues (3), whereas Krogh and Lindhard ascribed it to errors inherent in the Haldane-Priestley method of collecting alveolar air (5). Neither of these views has been established (6–8). However, in calculating the various pulmonary air fractions it has been generally assumed that gaseous exchange does not occur in the respiratory dead space.

The studies to be reported were undertaken to ascertain by direct measurement whether rapid transfer of oxygen and carbon dioxide occurs in the respiratory dead space and if so what bearing it might have on aspects of pulmonary function calculated from analyses of expired and alveolar air.

METHODS

The studies were carried out in 2 healthy adults at rest in a recumbent posture one to two hours following breakfast. A small rubber catheter with two holes, each 3 mm. in diameter within 2 cm. of the distal end, was threaded through one nostril until the distal hole was visible just below the uvula. A standard metal three-way stopcock inserted into the proximal end of the rubber catheter served to join it to a mercury gas sample tube evacuated to approximately 40 cc. and to a 20-cc. luer-lok syringe filled with room air. The nostrils were occluded securely with a nose clip. With a rubber mouth piece in place, the subject inhaled room air and exhaled into a Tissot spirometer. The rate and depth of respiration were

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recorded on an ink-writing drum attached to the spirometer. During the inspiration just preceding the collection of an air sample the catheter, which had a capacity of 2 cc., was flushed with 15 cc. of room air from the syringe attached to the three-way stopcock. At the moment when the subject completed the inspiration he was ordered to hold his breath and perform a Valsalva maneuver. At the same time he occluded the open end of the rubber mouth piece with his tongue. These maneuvers assured collection of air samples from the supraglottic portion of the respiratory dead space. Air samples were then collected in the evacuated mercury tube either immediately (within 1 or 2 sec.) or at any desired interval up to 40 seconds. In several instances air samples were gently drawn in four 10 cc. portions into an air-tight syringe and transferred to a mercury tube to learn whether the strong suction exerted by the evacuated mercury tube introduced a source of error.

The momentary pause in respiration, necessitated by the collection of air from the supraglottic portion of the respiratory airway, did not alter the pattern of respiration during the few minutes that it was recorded immediately thereafter. In 11 instances expired air was collected for approximately three minutes prior to collection of the supraglottic air sample and for three to four minutes thereafter.

Gas samples were analyzed in duplicate in a Haldane analyzer and results were compared with the composition of room air, which was found to contain consistently 20.93 ± 0.04 volumes per cent oxygen and 0.03 ± 0.03 volumes per cent carbon dioxide. The room was well ventilated with outdoor air.

RESULTS

A definite but small rise in carbon dioxide tension and fall in oxygen tension of inspired room air occurs in the supraglottic portion of the respiratory dead space, when the breath is held for one to two seconds after the end of inspiration while the subject performs a Valsalva maneuver (table 1). The average rise in carbon dioxide tension in 30 observations was 3.7 mm. with a standard deviation of 1.8 mm. Hg. The average fall in oxygen tension for a similar number of observations was 3.7 mm. Hg, with a standard deviation of 2.3 mm. Hg. The close agreement between the results obtained from analysis of air samples drawn in one 40-cc. portion into an evacuated mercury tube and those of similar volume drawn by syringe in 10 cc. portions indicates that with the former method, which was used in most instances, air was not drawn from poorly ventilated stagnant areas. When the breath is held for progressively longer periods, the proportion of carbon dioxide increases at a nearly steady rate whereas all of the decrease in the proportion of oxygen appears to occur within at most 10 seconds (fig. 1). Though there is considerable daily variation in the rate of exchange of these gases for similar periods of breathholding, the trend is consistent and it is not related to the depth of breathing, minute volume of respiration or respiratory quotient based on analysis of expired air.

DISCUSSION

Analyses of air samples drawn from the respiratory dead space at the end of either inspiration or expiration should indicate whether or not the dead space is an inert

TABLE 1. DATA CONCERNING STUDIES ON EXCHANGE OF CARBON DIOXIDE AND OXYGEN IN THE
SUPRAGLOTTIC PORTION OF THE RESPIRATORY AIRWAY WITHIN 1 TO 2 SECONDS AFTER THE END
OF INSPIRATION

OBSERVA-TION	TIDAL AIR[1]	VENTILA-TION RATE[1]	SUPRAGLOTTIC AIR				EXPIRED AIR		RQ
			CO_2	O_2	pCO_2	pO_2	CO_2	O_2	
			Content		Rise	Fall	Content		
	cc.	l/min.	vol. %		mm. Hg.[2]		vol. %		
1	401	6.4	0.57	20.49	3.9	3.0	3.87	16.88	.94
2	352	8.5	0.53	20.45	3.6	3.4	3.32	17.39	.93
3	374	8.6	0.77	20.29	5.3	4.3			
4	470	5.2	0.34	20.59	2.2	2.5	4.15	16.43	.90
5	570	5.1	0.23	20.80	1.4	0.7			
6	410	3.3	0.43	20.65	2.8	1.8			
7	285	4.6	0.33	20.63	2.2	2.2	3.35	17.22	.90
8	280	3.9	0.42	20.56	2.8	2.6			
9	642	6.4	0.57	20.47	3.9	3.1			
10	708	5.7	0.30	20.64	2.0	2.2	4.06	16.29	.84
11	764	5.3	0.72	20.19	4.9	5.4	3.99	16.01	.77
12	821	5.7	0.44	20.49	2.9	3.2			
13	622	5.0	0.35	20.66	2.3	1.9			
14	697	5.6	0.37	20.60	2.4	2.3	4.16	16.17	.84
15	693	4.9	0.28	20.71	1.8	1.5			
16	662	5.3	0.28	20.66	1.8	2.0			
17	526	4.7	0.30	20.71	1.9	1.5			
18	681	6.1	0.19	20.75	1.1	1.3			
19	880	7.0	0.98	19.95	6.8	7.2	3.55	16.91	.85
20	680	4.1	0.94	20.00	6.6	6.7			
21	870	4.4	0.74	20.27	5.1	4.7			
22	740	5.2	0.86	20.01	6.0	6.9	3.94	16.14	.78
23	750	5.3	1.19	19.56	8.3	10.3			
24	880	6.2	0.53	20.42	3.6	3.7	3.70	16.68	.83
25	864	5.2	0.37	20.59	2.4	2.4			
26	896	5.4	0.53	20.39	3.6	3.9			
27	670	4.0	0.56	20.36	3.8	4.1			
28	838	5.0	0.80	20.00	5.5	7.0			
29	902	6.3	0.33	20.67	2.1	1.9			
30	948	6.5	1.12	19.71	7.8	8.7	3.79	16.58	.83
Average........................					3.7	3.7			
Standard Deviation.........................					1.8	2.3			

[1] Expressed at body temperature, ambient pressure, saturated.

[2] Calculated from inspired air containing 20.93 ± 0.04 vol. % O_2 and 0.03 ± 0.03 vol. % CO_2 at vailing barometric pressure less aqueous tension, 47 mm. Hg.

conduit.   Collection of a sample of air at the end of inspiration offers however a better standard of comparison (inspired room air) than does expiration (expired air).

It seems likely that the gas exchange occurs across the mixed glandular secretions which bathe the supraglottic area.   By far the greatest portion of the secretions

comes from the salivary glands. It has been demonstrated that the nitrogen (9) and carbon dioxide content of saliva reflect closely the level of these gases in blood, that mixed saliva generally has a *p*H of 6.5 to 6.8 and that it contains oxygen (10). The circumstances most favorable for transfer of these gases, between the layer of mixed secretions and the air in the respiratory airway, would appear to be present at the end of inspiration when the tension of carbon dioxide in the airway is at its lowest level and the tension of oxygen is at its highest.

These studies establish what had previously been inferred by Henderson concerning output of carbon dioxide into the respiratory dead space (1). They also demonstrate that oxygen uptake of approximately the same magnitude as carbon dioxide
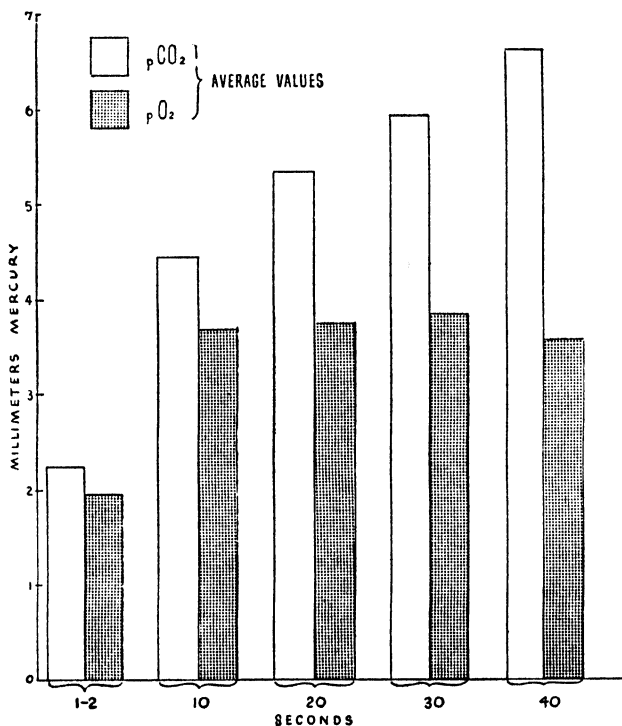
Fig. 1. GAS EXCHANGE in supraglottic portion of respiratory airway. Average tension values for breathholding are based on serial determinations carried out on different days. There were 6 observations at 1–2 sec., 5 at 10, 20 and 30 sec. and 4 at 40 sec. The range of values in mm. Hg of carbon dioxide and oxygen tension respectively was: at 1–2 sec., 1.7–2.8, 1.5–2.9; 10 sec., 3.7–5.9, 2.9–5.3; 20 sec., 4.5–7.1, 2.7–5.9; 30 sec. 5.0–8.4, 2.2–6.7; 20 sec., 6.5–6.9, 2.1–5.1.

output occurs in the supraglottic portion of the respiratory dead space during quiet respiration. The transfer of these gases cannot, therefore, account for a difference between the respiratory quotient as measured in expired and alveolar air since the ratio of exchange is approximately one.

An average rise in carbon dioxide tension and fall in oxygen tension of 3.7 mm. Hg occurs in the supraglottic portion of the respiratory airway (table 1). It is generally believed that in healthy subjects at rest, the carbon dioxide tension of inspired air rises 40 mm. Hg (from 0 to 40 mm. Hg) and oxygen tension of inspired air falls 50 mm. Hg (from 150 to 100 mm. Hg) in the alveoli. This fraction represents approximately 9 per cent of the total carbon dioxide output and 7 per cent of the total oxygen consumption of the total gaseous exchange in the lungs.

The studies during breathholding bring out a distinct difference between exchange of carbon dioxide and oxygen (fig. 1). Carbon dioxide concentration increases steadily during breathholding whereas the concentration of oxygen falls only within approximately the first 10 seconds. The findings are consistent with Henderson's observation that with breathholding there is a greater decrease in the size of the respiratory dead space calculated for carbon dioxide than for oxygen (1). It would appear that the difference in exchange is due to the much more rapid rate of diffusion of carbon dioxide through body tissues and fluids and to the limitations imposed on oxygen exchange by its absorption coefficient in the mixed glandular secretions which bathe the supraglottic portion of the respiratory airway.

SUMMARY

An exchange of carbon dioxide and oxygen of approximately the same magnitude occurs in the supraglottic portion of the respiratory dead space within one to two seconds after the end of inspiration in healthy subjects at rest. This exchange does not account for any difference reported in the *RQ* of expired and alveolar air. When the breath is held at the end of inspiration for progressively longer periods of time, the carbon dioxide concentration increases at a nearly steady rate, whereas the oxygen concentration falls only during the first 10 seconds.

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THE AMERICAN PHYSIOLOGICAL SOCIETY
PROCEEDINGS
First Fall Meeting
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The following are abstracts of papers presented at the First Fall Meeting of The American Physiological Society, held at the University of Minnesota, Minneapolis, and at the Mayo Foundation and Mayo Clinic, Rochester, Sept. 16–18, 1948.

Hormonal alterations in men exposed to heat and cold stress. RICHARD A. BADER, HAROLD J. STEIN, JOHAN W. ELIOT AND DAVID E. BASS (introduced by H. S. BELDING). Quartermaster Climatic Research Laboratory, Lawrence, Mass.

Hormonal alterations were measured in three men who were subjected to heat and cold stress utilizing intermittent exposures to heat (107°F. dry bulb; 89°F. wet bulb) and to cold (−20°F.) as stress stimuli. The number of circulating eosinophils, which can be depressed by anterior pituitary adrenocorticotropic hormone (ACTH) or Compound F, were significantly reduced in daily counts during (1) exercise, (2) a combination of heat and exercise, and (3) a combination of cold and exercise, compared to a baseline period without stress. Adrenal cortical reserve was assessed by the response to ACTH in the manner proposed by Forsham *et al.* (*J. Clin. Endocrinol.* 8: 15, 1948), following the heat, and again following the cold exposures. These studies showed a slightly 'abnormal response' in one individual and an essentially 'normal response' in the other two subjects. This suggests that the heat and cold stresses were not sufficient to deplete the adrenal cortical reserve as measured by ACTH. The heat stress was nevertheless, sufficient to produce acclimatization, although definite acclimatization to cold was not demonstrated. No significant changes were found during any of the stress periods in the following indices: urinary uric acid-creatinine ratio, urinary 17-keto-steroid excretion, absolute lymphocyte counts, and basal metabolism.

Influence of various diuretic substances on the renal excretion of electrolytes in the dog. DAVID BALDWIN, A. P. CROSLEY, JR., AND P. J. TALSO (introduced by ROBERT W. CLARKE). Medical Dept. Field Research Laboratory, Fort Knox, Ky.

Unanesthetized dogs have been studied during the administration of four diuretics. Inulin and PAH clearances were measured. Sodium and potassium were determined by flame photometry. Intravenous hypertonic saline infusion, which raised the serum sodium and glomerular filtration, reduced tubular sodium reabsorption from 98% to 80% of the filtered amount. The fractional tubular reabsorption of potassium was much more severely depressed than that of sodium. Intravenous aminophyllin raised glomerular filtration but did not alter serum sodium. Tubular reabsorption of sodium rose in proportion to filtration. Potassium excretion was augmented proportionally more than sodium, tubular reabsorption of potassium remaining constant. Mersalyl given by slow intravenous injection caused the tubular reabsorption of sodium to fall relative to the slightly elevated rate of glomerular filtration and to the tubular load. In spite of a slight increase in the tubular load of potassium the rate of its reabsorption fell. The fraction of filtered potassium which the tubules reabsorbed was reduced

but by a different means than that shown with aminophyllin. Intravenous hypertonic mannitol infusion somewhat decreased filtration, and reduced serum sodium and potassium slightly. Sodium reabsorption also fell, to a greater extent than the load, and the fraction of the latter which was reabsorbed was significantly depressed. There was a great fall in potassium reabsorption, 25% of the load being excreted. When more sodium is filtered through the glomeruli it is reabsorbed by the tubules in preference to potassium. The diuretics studied decreased the relative tubular reabsorption of potassium far more than of sodium.

Total gastrectomy in the rat. DONALD C. BALFOUR, JR., AND GEORGE M. HIGGINS. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

The effect of total gastrectomy has been studied extensively in the larger animals such as the dog, cat, pig, monkey and also in man. It has been shown that after gastrectomy it is difficult to maintain normal weight, a secondary anemia will usually develop and a homogeneous osteoporosis can often be demonstrated. Nutritional studies have been done on the totally gastrectomized rat. It is found that the gastrectomized rat is incapable of maintaining adequate food intake over a long period of time. By selestine diet studies it was shown that the gastrectomized rat selects a diet which is lower in protein content than the diet of the normal rat. Replacement therapy with gastric extracts does not improve the nutrition of the animal and injections of liver extract and vitamin B_1 improve it only temporarily.

Influence of prolonged administration of thiouracil on growth and metabolism. S. B. BARKER. Dept. of Physiology, State Univ. of Iowa, Iowa City.

Fertility is markedly reduced in adult female rats rendered hypothyroid by thiouracil inhibition of the thyroid gland, an observation which may be explained on the basis of altered estrus cycles. Cretinoid young have been obtained by procedures involving early depression of thyroid function with thiouracil, and have been maintained at a stage of incomplete development for periods up to 16 months by continuing the thiouracil administration. These animals have large, colloid-free thyroids, low plasma PI values and B.M.R.'s lowered by 20 to 35%, further confirming the lowered thyroid activity. Even after 16 months of depressed thyroid function, withdrawing the thiouracil results in a temporary renewal of growth, plus recovery of the lost reproductive capacity in some of the animals. The long-inhibited gland recovers its ability to store thyroglobulin and to release into the blood stream enough thyroid hormone to cause the B.M.R. to return to normal even though the plasma level of hormone is not brought completely to normal. Reproductive activity is rapidly restored, and apparently normal offspring are produced by females with weights plateaued at less than half the normal value.

Relation of the adrenal glands to the renin concentration of the canine kidney. HERBERT E. BESSINGER (by invitation) AND GEORGE E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Study was made of the renin concentration of the kidneys of 21 dogs, three to eight weeks following unilateral renal artery constriction. Three of the dogs were bilaterally adrenalectomized and maintained by salt therapy only. The condition of adrenal insufficiency did not appear to reduce the renin concentration of the renal artery constricted kidney determined four weeks after unilateral constriction, but appeared to favor renin return to the contralateral non-constricted kidney at which time it normally contains no (or nearly no) renin. The absence of both adrenal glands in 3 bilaterally adrenalectomized dogs with unilateral renal artery constriction receiving maintenance doses of desoxycorticosterone acetate (Schering) does not alter the low or absent renin content of the contralateral, non-constricted kidney six to eight weeks after renal artery constriction. The injection of DCA in nine unilaterally renal artery constricted dogs for periods of three to six weeks following renal artery constriction significantly decreased the extractable renin from the constricted kidney, but did not change the low or absent renin concentration of the contralateral, non-constricted kidney either three or six weeks after unilateral renal artery constriction. The renin was significantly decreased in the kidneys of three normal dogs receiving DCA for five weeks.

Renal tubular excretion of N-methylnicotinamide by the dog. KARL H. BEYER, HORACE F. RUSSO, S. R. GASS, KATHARINE B. MILLER AND ALICE A. AMAN. Dept. of Pharmacology, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa.

In the abstracts of communications of the XVII International Physiological Congress (p. 217, 218) Sperber indicated that N-methyl-nicotinamide (NMN) was excreted by the tubules of the chicken kidney. Since this is the first instance of the tubular excretion of a base, other than possibly creatinine, we undertook to examine its excretion in dogs. Using the analytical method of Huff and Perlzweig (*J. Biol. Chem.* 167: 157, 1947) we found that the renal clearance and the extraction of N-methyl nicotinamide from the renal blood

flow was greater than for creatinine, indicating tubular excretion of the NMN. As the plasma concentration of NMN was elevated its clearance was depressed to approach glomerular filtration rate. The clearance of NMN was not depressed by p-aminohippurate (PAH) or by caronamide. Therefore it was concluded that the tubular transport mechanism for NMN was separate and distinct from the mechanism responsible for the tubular excretion of PAH. Also, that caronamide which is capable of inhibiting the organic acid tubular excretory mechanism is not capable of inhibiting the excretion of this strong base. Apparently there exists in the mammalian tubules (dogs) a transport mechanism for the renal tubular elimination of organic bases.

Thresholds for production of seizures by photic stimulation in man. R. G. BICKFORD (introduced by E. H. LAMBERT). Dept. of Physiology, Mayo Foundation, Rochester, Minn.

Previous studies in this laboratory have shown that approximately five % of epileptic patients are photosensitive. Minor seizures may be induced in this group of patients by subjecting them to flickering light of the requisite intensity. By controlling the intensity of the light stimulus it is possible to make measurements of the convulsive threshold. Three light sensitive subjects were used. They were subjected to 15-second periods of stimulation by light from a reflecting type photoflood bulb at a distance of 3 feet. The beam was interrupted nine times per second by a rotating $\frac{3}{4}$ blackened sectoring disc. The intensity of the light stimulation was controlled by changing (by means of a variable transformer) the voltage across the photoflood bulb in 10 volt steps between 20 and 110 volts. This represents a brightness range from 3 to 1200 ft candles. A 15-second rest period is given between each stimulation. The convulsive threshold is the lowest light intensity (or voltage) which will induce a seizure within the stimulus period. The occurrence of the seizure is detected by observation of clinical signs and electroencephalographic recording of the seizure discharge. In the resting patient the convulsive threshold may remain constant for several hours if the testing is not repeated at less than 15-minute intervals. Occasionally a slowly progressive increase or decrease in the threshold is observed. Barbiturate drugs in nonhypnotic doses raise the convulsive threshold. Amytal, which is the most effective of the group, may prevent seizures occurring at the highest light intensity. Tridione is less effective. Bromides even in hypnotic doses are without effect. Benzedrine and metrazol lower the convulsive threshold.

Inhibitors of gastric secretion occurring in gastric juice and gastric mucin. CHARLES M. BLACKBURN AND CHARLES F. CODE. Mayo Foundation, Rochester, Minn.

Using an assay method developed by Code, Blackburn, Ratke and Livermore for the determination of inhibitors of gastric secretion, a study has been made of the inhibitors occurring in human gastric juice. The observation by Brunschwig and his co-workers that there is an inhibitor in achlorhydric gastric juice of patients who have pernicious anemia has been confirmed. The observation of Brunschwig and his associates that the inhibitor is present in achlorhydric gastric juice from patients without other demonstrable changes in gastric function has also been confirmed. Brunschwig and his collaborators tested for the inhibitor by injection of the juice or extracts of the juice into dogs with gastric pouches when these were secreting in response to the ingestion of a meal. We have found that the gastric secretory inhibitor present in human gastric juice also inhibits the secretion of Heidenhain pouches in dogs when these pouches are stimulated by repeated injections of histamine. In a search for the source of the inhibitors present in human gastric juice, we have tested gastric mucin and gastric pepsin. We have found that intravenous injection of gastric mucin produces a pronounced reduction in the secretion of juice from Heidenhain pouches in dogs when these are stimulated by histamine. The preparations of pepsin so far tested have not contained an inhibitor.

Stimulating effect of carbohydrate, fat and protein meals on duodenal secretion. D. BLICKENSTAFF (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Carbohydrate, fat and protein test meals were fed to mongrel dogs, some with transplated pouches of the upper duodenum, fashioned according to the method used by Florey and Harding, and Sonnenschein, Grossman, and Ivy, and others with a similar preparation except that the pouch was opened to form a rectangular flap. The following foodstuffs were used as test meals: 50 grams of dextrose in 100 cc. of water, 50 cc. cottonseed oil, and 50 grams of lean beef. The duodenal secretion was collected by means of a funnel into a graduated centrifuge tube. Results of 32 experiments, compared with control experiments using 50–100 cc. saline, show clearly that the duodenal glands respond to all three types of 'pure' meals by increasing their rate of secretion. A slight difference in the responses of the two types of preparations suggests that part of the increased secretion in the 'pouch' type may be dependent on motor activity which causes rubbing together of the

mucosal folds and incidental mechanical stimulation. The conclusion is drawn that there is no single specific absorbed secretagogue controlling Brunner's glands, which strengthens the supposition that the humoral agent described as having this property is a hormone.

Alkaline phosphatase activity of the developing egg and embryo of the grasshopper, Melanoplus differentialis. JOSEPH HALL BODINE AND LAURENCE ROCKWELL FITZGERALD. State Univ. of Iowa, Iowa City.

Alkaline phosphatase activity of eggs and embryos of the grasshopper, *Melanoplus differentialis*, of various ages was determined, using a modified King-Armstrong procedure. ·The activity is expressed as μg. phenol liberated in 30 minutes per egg (or per embryo). During the first 10 days of development, there is no detectable phosphatase activity in the grasshopper egg. Between the 10th and 21st days of development the activity increases from 0 to about 11. At this time, diapause sets in, but despite the developmental block, the activity doubles by the 80th day, reaching about 22 at this time. If the diapause is broken, the activity increases during the postdiapause period, reaching a level of about 36 at the time of hatching. There is no detectable activity in the embryo proper until the 8th day of postdiapause development (about 10 days before hatching), and this remains very low (2–3) until the time of hatching, at which time the nymph contains all the activity of the original egg. Experiments in which the eggs were centrifuged show that as the embryo becomes packed into the centrifugal pole of the egg, displacing the extra-embryonic fluid, the activity of the centrifugal portion of the egg decreased, and the activity of the central portion increased, as compared with similar sections of an uncentrifuged egg. Experiments in which the extra-embryonic fluid was separated from the embryo, yolk, shell, etc., show that nearly all the activity of the whole egg is to be found in the extra-embryonic fluid. This indicates that the alkaline phosphatase, like tyrosinase, may be formed by the serosa, is found in the extraembryonic fluid, and is incorporated into the embryo at the time of hatching when the embryo swallows this fluid.

Electrical stimulation of the central nervous mechanism for vomiting in the cat. HERBERT L. BORISON (introduced by S. C. WANG). Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.

Vomiting has been elicted in 10 out of 20 cats in which the region of the calamus scriptorius in the brainstem was stimulated faradically with a bipolar needle electrode oriented by means of the Horsley-Clarke stereotaxic instrument. Decerebrate preparations were used in all experiments since anesthesia severely depresses emesis. The graphic response, as recorded with thoracic and abdominal pneumographs, is characterized by an apneusis with vomiting occurring at the peak of inspiration. The main criterion for the elicitation of vomiting, however, was the expulsion of vomitus consisting of milk curd. Stimulating current thresholds for the vomiting response have been determined such that at a constant frequency of 50 impulses per sec., the threshold of intensity is about 8 volts and at a constant voltage of 12 volts, the frequency threshold is between 33 and 50 impulses per sec. By histological identification of the reactive points, the responsive region for emesis has been localized to the area corresponding to the tractus solitarius and the gustatory nucleus and a small portion of the reticular formation lying ventral to these structures. The vomiting center bears a close topographical relationship to the salivatory center (*J. Neurophysiol.* 6: 195, 1943), the spasmodic respiratory center (*Federation Proc.* 7: 10, 1948), and the inspiratory center (*Am. J. Physiol.* 126: 673, 1939). This illustrates the anatomical proximity of the component parts involved in the expression of a complex physiological pattern.

Effect of testosterone propionate on the total urinary nitrogen excretion of the rat following burns. JOHN W. BRAASCH AND GEORGE E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

The total urinary nitrogen excretion of adult male and female albino rats was followed after an approximately 33% deep second or third degree thermal burn. Constancy of intake was assured by daily force feeding of 22 cc. of a liquid diet made up of 73% carbohydrate, 18 % protein, and 10% fat and yielding 2.9 calories per cc. Each sex was divided into three groups of approximately 6 animals each. Two groups were burned, one receiving daily injections of 2.0 mg. of testosterone propionate in peanut oil and the other just peanut oil. In the case of the males, the administration of the drug was begun at the time of the burn. In the case of the females, it was started 2 days before the burn. In addition, one group of both males and females was just injected daily with 2.0 mg. of testosterone propionate in peanut oil. As was anticipated, the urinary nitrogen of the peanut-oil treated, burned rats was elevated for approximately 6 days after a temporary 2- to 3-day depression following the burn. In those animals treated with testosterone propionate, the level of urinary nitrogen excretion remained approximately at the control value. The effect of testosterone propionate on the nitrogen excretion of normal male and female rats was approximately the same for both sexes and also

equal to the calculated effect in the burned animals for 8 to 10 days after the burn. On days 10 to 12 following the burn, the effect of testosterone propionate is no longer evident whereas in the unburned animals, the effect continues unabated.

Tissue distribution with time following single intravenous administration of sodium pentothal (sodium ethyl (1-methylbutyl) thiobarbiturate). L. M. BROOKS, J. L. BOLLMAN, E. V. FLOCK AND J. S. LUNDY. Mayo Foundation and Clinic, Rochester, Minn.

We have made a study, using the method of Jailer and Goldbaum (with slight modifications) and also using radioactive pentothal, S^{35}, to determine the distribution of pentothal at various times after a single intravenous injection of amounts of the drug which produce deep anesthesia in normal rats. Male white rats weighing approximately 200 gm. were given 40 mg/kg. of pentothal into the saphenous vein. One minute after the start of injection 4 to 5 cc. of blood were withdrawn by direct cardiac puncture, the heart excised to stop circulation, and the other organs excised thereafter as rapidly as possible. Other rats were similarly injected and subsequently killed for plasma and tissue analysis 3, 10, 30, 60 or 120 minutes after injection. From our results it is apparent that pentothal is rapidly distributed throughout the body and that the greatest concentration in each tissue is reached within one minute. There is no evidence of subsequent marked accumulation in any tissue studied or of any special affinity for pentothal by any tissue. The concentration in the major tissues in not markedly different from that of the plasma at any time, that found in the liver and kidneys being somewhat higher than that of the plasma and that of the brain, muscle, lung and intestine being approximately equal but slightly less than that of the plasma. Following the immediate distribution of pentothal there is a subsequent slow decline in the amount found in each tissue, this loss from each tissue being at approximately the same rate.

Survival time of dogs at a barometric pressure of 30 mm. Hg. B. H. BURCH (by invitation) AND F. A. HITCH-COCK. Laboratory of Aviation Physiology, Ohio State University, Columbus.

Two series of unanesthetized dogs were explosively decompressed at a rate of 33,650 mm. Hg/sec. and maintained for varying lengths of time at a terminal pressure of 30 mm. Hg. In the first series the animals were exploded from 700 mm. Hg, (2500 ft.), while in the second series, the explosion was from 180 mm. Hg (35,000 ft.). These animals were maintained on 100% O_2 at this altitude for at least 20 minutes preceding the explosion. The initial alveolar pO_2 was thus essentially the same in both series. Of the 13 dogs making up the first series, none survived the terminal altitude for 80 seconds or longer. In series 2 (17 dogs) no mortality occurred when the duration at terminal altitude was less than 100 seconds, 50% mortality occurred in 100 seconds, and 100% mortality when exposure to terminal altitude was 180 seconds or longer. Recovery in *series 2* was faster and more complete than in *series 1*. A series of experiments designed to determine the immediate cause of death of animals exposed to pressures of 30 mm. or less have been carried out. In anesthetized animals, the anterior chest wall was replaced by a plastic window and observations made on the heart. Fibrillation occurred in about 80 seconds after explosive decompression. Observations made through plastic windows sutured into the right ventricle showed the presence of bubbles in the right ventricle and in the coronary vessels within 35 seconds after the explosion.

Reproducibility of values for arterial oxygen saturations under varying conditions in a patient with an intracardiac venous arterial shunt. H. B. BURCHELL AND E. H. WOOD. Mayo Foundation, Rochester, Minn.

Direct and oximetric studies of arterial oxygen saturation have been carried out at approximately monthly intervals for ⸱ period of a year on an adult patient with a congenital cardiac defect of the cyanotic type. During supine rest the arterial oxygen saturation determined by Van Slyke analyses averaged 85.0 % and ranged from 81.4 to 88.7%. During this period the oxygen capacity decreased from 33.6 to 20.9 volumes % as a result of monthly phlebotomies. The arterial oxygen saturation (Van Slyke) during the last 60 seconds of a 5.5-minute period of walking at 1.7 miles per hour averaged 65.5 and ranged from 59.3 to 73.4%. The resting arterial oxygen saturation increased on the average to 94.5% and ranged from 92.5 to 95.9 during breathing 100% oxygen. The evidence obtained by means of cardiac catheterization, carried out on two occasions, indicates a constancy in the venous shunt during these conditions. When walking at 1.7 miles per hour, the arteriovenous difference increased and the fall in arterial oxygen saturation was largely related to decreased oxygen content of the venous blood shunted to the arterial side and not to any increase in the percentage shunt. The studies on this and similar individuals apparently indicate that in patients which intracardiac venous arterial shunts who are well compensated from the circulatory viewpoint, the percentage of venous arterial shunt may be relatively constant and that the result of direct and oximetric arterial oxygen saturation studies during supine rest, standing, walking and breathing 100% oxygen are relatively reproducible.

Relation between pressure and flow in the perfused frog's leg. ALAN C. BURTON. Dept. of Biophysics, Univ. of Western Ontario, London, Canada.

Whittaker and Winton, Green *et al.*, Roome, Pappenheimer and Maes, and others have carefully investigated the pressure-flow relations in the limbs of mammals. The distensibility of the vessels, and the anomalous viscosity of blood both complicate the interpretation. By the aid of a micro-flowmeter, modified from that of Bozler, the relation has been studied, especially very low pressures, in the Trendelenburg preparation. Flow and pressure are linearly related, but all flow ceases when the pressure falls below a critical value of about 5 cm. of Ringer's solution. When the pressure is lowered abruptly from a high value to below this critical value, reverse flow occurs, while the vessels empty and force fluid against a pressure gradient. This indicates a 'residual tension' in the walls of the smallest vessels, given by the equation $T = Pr$. This tension is not due to tissue pressure, and probably not to elastic or smooth muscle tension. It appears to be an interfacial tension between Ringer's solution and the walls, since it is removed by adding wetting agents (bile salts etc.) to the solution.

Effect of periadrenal ligation on blood pressure of the dog. ROBERT G. CANHAM (by invitation) AND GEORGE E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Unilateral, subtotal periadrenal ligations were performed in 9 dogs and blood pressures were followed for one to five months. Six of these dogs were then subjected to similar ligations of the contralateral adrenal; in addition a one-stage bilateral periadrenal ligation was carried out in another dog and blood pressures were followed as before. Finally, in 6 of the original 10 dogs unilateral renal artery constriction with a Goldblatt clamp and contralateral nephrectomy was performed. Aside from one dog which showed an average rise in pressure of 21 mm. Hg following unilateral ligation and 36 mm. Hg following bilateral ligation, none of the animals developed hypertension. Hypertension, however, was produced later by the Goldblatt method in 4 out of 6 dogs that had previously been subjected to periadrenal ligations.

Γ-dichroine, the antimalarial alkaloid of Chang Shan. K. K. CHEN. *Div. of Pharmacology, Lilly Research Laboratories, Indianapolis, Ind.*

γ-dichroine is a new alkaloid isolated by Chou (*J. Am. Chem. Soc.* 70: 1765, 1948) from Chang Shan, *Dichroa febrifuga.* Its Q value in ducklings against *Plasmodium lophurae* was estimated to be about 148; and in canaries against *P. relictum*, 137. Dr. L. H. Schmidt of Christ Hospital, Cincinnati, observed that

γ-dichroine in the dose of 0.4–0.8 mg/kg. definitely reduced the parasitemia in rhesus monkeys infected with *P. cynomologi* (personal communication). The LD$_{50}$ in mice was 10 ± 0.5 mg/kg. intravenously, and 2.74 ± 0.41 mg/kg. orally. By repeated administration in mice, γ-dichroine caused hydropic degeneration of the liver in certain animals. Vomiting occurred when γ-dichroine was intravenously injected into pigeons. Diarrhea appeared in rabbits when the alkaloid was similarly administered. In anesthetized dogs, γ-dichroine in the dose of 2.5 mg/kg. by vein induced an increase of duodenal peristaltic activity, but had no effect on respiration and blood pressure.

Further evidence on conversion of CO to CO_2 by tissues including experiments with radioactive carbon. ROBERT T. CLARK, JR., J. NEWELL STANNARD AND W. O. FENN. Dept. of Physiology and Vital Economics and Atomic Energy Project, Univ. of Rochester, Rochester, N. Y.

Previous studies from this laboratory have indicated that the metabolism of resting heart and skeletal muscle was increased 2 to 3 times when the air surrounding the tissues was replaced by a mixture of 80% and 20% O_2. Evidence has been presented that this stimulation of respiration was due to the burning of CO to CO_2 by the tissues. In other tissues such as nerve, stomach, liver, and kidney the metabolism was increased only slightly, and in skin there was some inhibition of the normal respiration. In the present investigation the respirometer experiments were carried out as before with the exception that CO containing radioactive carbon ($C^{14}O$) was used in place of the usual CO in the CO-O_2 mixture. Utilizing this procedure it was possible to measure the CO consumed by the tissues in two ways, 1) by determining the excess respiration in the respirometer and 2) by measuring the radioactivity of $C^{14}O_2$ of the respired gases after absorption in $Ba(OH)_2$. Such determinations were made on frog heart, skeletal muscle, skin and nerve. The results show that the amount of CO consumed, as calculated from the radioactivity of the CO_2 collected, agreed with that from the volumeter data, thus proving that the stimulation is primarily due to the burning of CO to CO_2. Also, the rate of burning by each tissue studied was in the same direction as previous studies have shown. The rate was very high in heart and skeletal muscle, low in nerve, and negligible in skin.

Quantitative determination of inhibitors of gastric secretion. CHARLES F. CODE, GEORGE R. LIVERMORE (by invitation), HENRY V. RATKE (by invitation) AND CHARLES M. BLACKBURN (by invitation). Mayo Foundation and Clinic, Rochester, Minn.

It was shown by Gray, Bradley and Ivy (*Am. J.*

Physiol. 118: 463–476, 1937) that the degree of inhibition of gastric secretion produced by enterogastrone is dependent to a considerable extent on the rate of secretion of gastric juice at the time of testing. This and the differences in size between dogs and stomachs or pouches of dogs have made quantitative comparisons of inhibitor activity somewhat difficult. The following procedure has been developed to overcome some of these difficulties and has been used satisfactorily to test some inhibitors. Dogs with Heidenhain pouches were used. The maximal rate of secretion of hydrochloric acid possible from these pouches in response to histamine given every 10 minutes subcutaneously was determined and checked repeatedly. Depending on the sensitivity desired, the inhibitor was injected intravenously while the pouches were secreting at a fourth of this maximal rate when a high degree of sensitivity was required, or at half of the maximal rate or greater if a less responsive, though somewhat more stable, preparation could be used. Administration of the dose of histamine giving the desired rate of secretion was contained until completion of the assay. As a rule the mean of the inhibition produced during the second and third hours after injection of the test substance was used in the calculation of the results. These may be expressed as percentage inhibition per weight or volume of inhibitor or in terms of some unit established for the particular study.

Hepatic and peripheral removal rates of the dog for intravenously injected bromsulphalein. CLARENCE COHN, RACHMIEL LEVINE AND MURIEL KOLINSKY. Dept. of Biochemistry, and Metabolism and Endocrinology, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

Bromsulphalein disappeared from the blood of eviscerated-hepatectomized-nephrectomized dogs more than three times as rapidly as Evans Blue which was injected intravenously at the same time. No evidence for excessive loss of BSP through the capillaries could be demonstrated in the eviscerate preparation. It was possible to estimate 'Pm' (maximal transfer capacity of the periphery for bromsulphalein), since it was impossible to 'saturate' extra-hepatic and extra-portal sites of bromsulphalein removal with serum levels as high as 100 mg. %. The rate of removal of intravenously injected BSP, being the sum of both portal and peripheral removal mechanisms, invalidates the use of the dye for measuring either hepatic blood flow or of ('Lm').

Studies on the in vitro secretion of HCl by the mammalian stomach. FRED E. COY, JR., (by invitation) AND WARREN S. REHM. Dept. of Physiology, Univ. of Louisville, Louisville, Ky.

In 1930 Delrue (*Arch. int. Physiol.* 33: 196, 1930) reported the *in vitro* secretion of HCl by the frog's stomach. In the present work we attempted to obtain *in vitro* secretion of HCl in the mammalian stomach. A piece of stomach, usually with the muscle layers dissected off, was tied around the end of a glass tube and the preparation then immersed in an appropriate fluid (Ringer's or serum) at 38°C., adjusted to a pH of 7.4 through which 95% and 5% CO_2 was bubbled. Saline (or tap water) was placed in the tube in contact with the mucosa and an oxygen mixture bubbled through this fluid. Twelve preparations of dogs' stomach, 12 of rabbits', 6 of hamsters', and 8 of guinea pigs' were used. The secretory rate was determined by draining the fluid in the tube and replacing with fresh saline, usually at 15-minute intervals. The pH of the saline from the tube was measured with a glass electrode. In only three cases did the pH of the saline fall below 5.0. Because of the possibility of an abnormally high CO_2 content of the tissue when 5% CO_2 was used, experiments were performed on guinea pigs with 100% O_2 and phosphate Ringer's. In 10 out of 12 experiments the pH fell into the 4's every 15 minutes for from two to several hours. In 4 experiments performed at 32°C. (see Davies, *Biochem J.* 40: xxxv, 1946) a higher secretory rate was obtained. The highest rate was one in which the pH dropped to 3.18 in 15 minutes.

Variations in a single subject of O_2 intake, acetylene minute volume, O_2 debt and RQ on 12 various exercises designed as tests of maximal circulatory capacity. THOMAS KIRK CURETON, JR., ALFRED W. HUBBARD (by invitation), LOUIS J. SAKAL (by invitation) AND W. KASTRINOS (by invitation). Univ. of Ill., Chicago.

In 15 tests of net O_2 intake on maximal exercises given to a 115 lb. meso-medial subject, of near average running endurance and 68″ tall, the range in net O_2 intake was from 1.620 l/min. to 2.780 l/min., S.D. about the mean ±.1865 and $\sigma_M = \pm.1154$. The highest minute volume was reached on the All-Out Step Test (40/min., 17″ bench) but the 7 mi/hr. Treadmill Run (8.6% slope) gave 25.25 l/min. A plot of $\dfrac{O_2 \text{ intake}}{.123}$ parallels the acetylene MV fairly closely, plotted against Cal/hr/kg., the rank order correlation being .58 but the true relationship is much better than this due to the curvilinear shape of the graph. The subject tensed up at 10 mi/hr. (8.6%) slope and the MV dropped to 18.76 l/min., a drop of 29.5%. The re-test variations in this work averaged 5.12% in net O_2 intake, although some of the tests were several weeks apart. The O_2 debt varied in 15 determinations on 12 different exercises from 3.38 l. to 10.93

l.; of the variations 1.965 l. and M ± .525 l. The more obvious causes of variation were 1) subject giving up before being 'All-Out', 2) variable recovery times (2) same exercises restricting ventilation. The 7, 10 and 13.5 mi/hr. All-Out treadmill runs (8.6% slope) caused O_2 debts of 9.54, 9.12 and 10.43 liters. The equation derived to fit the curve of relationship between the difficulty of the exercise in Cal/min. (X axis) and duration of exercise (Y axis) is: $Y = 16.01e^{-.088x}$ (between 20–60 Cal./min.). Above 60 Cal/min the graph is a straight line parallel to the base axis. The logarithmic plot shows all points practically on a straight line except for two exercises below All-Out capacity, one which obviously obstructed circulation (Burpec-Push-Ups) and one in which the subject stopped before exhaustion. The normal sitting RQ of .80 rose to 1.12 in All-Out exercise, +1.51 in the first bag of recovery gas and then dropped back to .80 or below. After three tests it went as low as .67 and after the 13.6 mi/hr. Treadmill Run it dropped to .56 at the end of training. The RQ is systematically lowered by training, the normal resting RQ dropping to .66. Several very highly trained endurance athletes have been found with RQ's as low.

Effects of microwave diathermy on the eye. L. DAILY, JR., K. G. WAKIM, J. F. HERRICK AND E. M. PARKHILL. Mayo Clinic and Foundation, Rochester, Minn.

A study was made of the changes in temperatures of the orbital tissues, aqueous, and vitreous humors and of the pathological changes resulting from exposure of the eye of the dog to microwaves. One series of acute experiments was performed on 36 dogs anesthetized with pentobarbital sodium. Thermocouple needles were used for recording temperatures of the orbit, aqueous and vitreous humors of the eye before and after exposure to microwaves. Exposure of the eye to microwaves for 30 minutes using 75% of the output with the corner director at a distance of 3 inches produced an average temperature rise of 1.9°C. in the orbit, 3.2°C. in the vitreous and 2.8° in the aqueous, exactly one minute after the microwaves were turned off. Another series of experiments was performed on 8 dogs similarly anesthetized and one eye was exposed to microwaves. Both eyes were studied clinically, including ophthalmoscopic examination of the media and fundi before and after exposure to microwaves. Eyes of 2 dogs that were exposed once daily, six and ten times respectively, to microwaves using 75% of the output with the director at a distance of 3 inches for 30 minutes, so far have shown no clinically observable pathologic findings. Eight 30-minute exposures of the eye of one dog once daily using 75% output with the director

at 2 inches produced anterior cortical cataract within six days after the last exposure. Seven similar exposures of the eye of another dog using 98% output with the director at 1 inch produced anterior cortical cataract within 24 hours after the last exposure. Over a period of 9 weeks these anterior cortical cataracts increased in size and density and then regressed and posterior cortical cataracts developed. In one dog, one 30-minute exposure of the eye to microwaves using 75% output with the director at 2 inches produced corneal clouding and partial iridoplegia within 24 hours after exposure. Microscopic examination of sections of this eye revealed round cell infiltration of the corneal stroma. Similar clinical and histologic changes were produced in the eye of another dog within 24 hours after two 30-minute exposures to microwaves, and in addition, vitreous opacities, reddening of the optic disc, whitening and elevation of the retina in the region the disc were observed ophthalmoscopically. Microscopic examination revealed red blood cells, leukocytes and fibrinous exudate in the anterior chamber with hemorrhage into the iris and ciliary processes. Necrosis of iris stroma and disorganization of the pigment layer of the iris and subchoroidal exudate and cystic degeneration of the retina were also observed.

Metabolism of the mouse stomach in vitro. HORACE W. DAVENPORT. Dept. of Physiology, Univ. of Utah, Salt Lake City.

The accumulation of acid in the lumen of the mouse stomach has been studied *in vitro*. It has been shown that carbonic, lactic and pyruvic acids account for only a small part of the total acid found, and the remaining acid is believed to be inorganic acid secreted by the parietal cells. Arsenite (0.34 mM) moderately inhibits acid secretion, but the amount of pyruvic acid accumulating in the presence of arsenite is one fourth the deficit in acid secretion. Addition of acetate does not reverse the arsenite inhibition. The total amount of pyruvic acid reduced and oxidized by the stomach is one fourth the amount of inorganic acid secreted. The use of pyruvic acid by the stomach is inhibited by 0.34 mM arsenite, but it is unaffected by 30 mg. % thiocyanate, a more potent inhibitor of acid secretion. Shay *et al.* (*Gastroenterology*, 6: 199, 1946) have shown that severe thiamin deficiency does not reduce total acid secretion in the rat. It is therefore tentatively concluded either that the oxidation of one mole of pyruvic acid must produce four moles of hydrochloric acid or that pyruvic acid metabolism is not an essential part of the acid secreting mechanism.

Validity and reliability measurements of inulin diodrast function tests. DEAN F. DAVIES (introduced by HENRY A. SCHROEDER). Section on Cardiovascular

Disease and Gerontology, National Institutes of Health, USPH, Baltimore City Hospitals, Baltimore, Md.

The validity of diodrast Tm was tested at different tubular loads by titration experiments and by repeated tests. In titration experiments 10 of 12 subjects showed a depression of Tm values when tubular load/Tm ratios were increased to an average of 4.81 after control periods during which tubular load/Tm ratios averaged 2.57. In tests repeated on different days the mean Tm values were significantly depressed when plasma diodrast levels were 20 per cent or more above control levels. An examination of reliability of these tests was made by determining reliability coefficients of single periods and of single tests of each function. An attempt was made to differentiate between deviations due to physiological variation and instrumental error by statistical means. It was found that instrumental error plays a negligible role in day-to-day variation. Perfect reliability would decrease the standard error of estimate of inulin clearance from 13.8 to 12.2 cc. plasma per minute, of diodrast clearance from 101.9 to 98.2 cc. plasma per minute, and of diodrast Tm by only 0.4 mg. diodrast iodine per minute. Validity of surface area correction was studied by a preliminary analysis of 37 adult male subjects between 29 and 89 years of age and surface areas ranging between 1.39 and 2.06 square meters. It showed that the correlation between surface area and diodrast clearances and between surface area and diodrast Tm is insignificant. On the other hand, the same individuals showed a significant correlation between basal metabolic rate and each of these functions.

Cochlear microphonics and action potentials in the guinea pig. HALLOWELL DAVIS, S. RIESCO-MCCLURE (by invitation) AND D. MCAULIFFE (by invitation). Central Institute for the Deaf, St. Louis, Mo.

Electric responses of guinea pigs' cochleas were studied with a three-channel oscilloscope. Electrodes were placed on round window and in small holes (less than 0.1 mm.) opening into scalae vestibuli, media or tympani in turns 1, 2 or 3. Escape of fluid was often avoided; when it occurred it usually initiated slow progressive failure of microphonics and action potentials. At all positions the action potential following a click was nearly identical. At a given position scalae media vestibuli gave the same microphonic responses. Scala tympani gave the same microphonic but reversed in polarity. The latency of each response was also the same (within 0.03 msec.) at all positions. The pick-up of microphonics seemed fairly localized because 1) input-output curves for pure tones differed with position in respect to location on sound-intensity

scale, maximum voltage, presence of 'overload,' and slope and shape of curve; 2) at high intensities the first *sub-harmonic* of frequencies above 3500 might be equal to the fundamental at turns 2 and 3 although barely detected at round window; and 3) interference effects between two tones might be quite different at turn 2 and round window. Nevertheless, the response at one position did *not* show any marked maximum or special sensitivity relative to frequency, provided the input was adjusted to keep the response at another position constant. There was merely a gradual increase in low-tone emphasis as the electrode approached the apex. With constant sound intensity there was a strong maximum (natural period) at 1700 cps.

Estimation of pulmonary capillary pressure. L. DEXTER, F. W. HAYNES, AND H. K. HELLEMS. Medical Clinic, Peter Bent Brigham Hospital and Dept. of Medicine, Harvard Medical School, Boston, Mass.

A cardiac catheter with the hole on the tip was introduced into a small branch of the pulmonary artery of dogs so as to obstruct the arterial lumen. Another catheter was introduced through the arterial system into the pulmonary vein so as to obstruct the venous lumen. Pressures were recorded with Hamilton and saline manometers. The pressure existing in the lumen of the artery distal to the obstruction is a result of the retrograde transmission of pressure from the next collateral branch entering the pulmonary artery. Anatomically, this is the pulmonary capillary bed. Physiologically, this is also the case since blood fully saturated with oxygen can be withdrawn through the catheter occluding the pulmonary artery. Pressures in the catheter obstructing the pulmonary vein average 4 to 6 mm. Hg higher than in that obstructing the pulmonary artery. The same held true when both pressures were raised by producing pulmonary embolism with lycopodium spores. When respiration was halted, there was little change in the relationship of the pressures, but when blood flow ceased (death), the pressures became equal indicating that the differences in the two pressures were largely a function of blood flow. It is concluded that the pressure recorded through the obstructed artery was less because blood flow was reduced locally and that through the obstructed vein greater due to passive congestion. Averaging of both pressures gives a means of estimating the absolute magnitude of pulmonary capillary pressure. Values obtained lay between 8 and 9 mm. Hg.

Man's ceiling as determined in the altitude chamber. D. B. DILL AND K. E. PENROD. Medical Div., Army Chemical Center, Md.

Experiments were carried out in the Aero-Medical

Laboratory, Wright Field, early in the war on how high man can fly breathing oxygen. This paper reports observations at 44,800 feet, the highest altitude maintained for any length of time without pressurization. The eight men exposed to this altitude from 15 to 44 minutes, exhibited a variety of respiratory adjustments. Pulmonary minute ventilation ranged from 15 to 56 l., arterial oxygen saturation, from 58 to 84% and arterial pH from 7.45 to 7.76. No one lost consciousness and only one became slightly tetanic. One of the 8 experienced after effects attributable to anoxia, headache, nausea and visual disturbances. All were in a state of imminent collapse. The calculated pressure gradient of oxygen from alveoli to arterial blood was zero in those with minimal alveolar ventilation but was 16 mm. Hg in the man with maximal ventilation (alveolar pCO_2 12 mm. Hg). In men with alveolar pCO_2 between 30 and 24 the ΔpO_2 ranged from 6 to 13. The failure to reach oxygen equilibrium in hyper-ventilation may reflect a reduction in time available for oxygen transfer, i.e., to faster blood flow through the pulmonary capillaries.

Studies of pulmonary function with the use of the oximeter.
J. C. DOUGLAS AND O. G. EDHOLM. Univ. of Western Ontario, London, Canada.

Blood in the left heart or systemic arteries contains fractions from well and poorly ventilated portions of lung which have come into equilibrium with each other. Therefore, under a given set of conditions, the mixed arterial saturation may be expected to reflect the adequacy of pulmonary function. Using the Millikan Oximeter as an indicator of arterial oxygen saturation, a study has been made of the increase in pO_2 of the inspired gas required to raise saturation to a given value (i.e. 100%) from its level of 95–98% when breathing room air. In normal subjects, an alveolar oxygen tension of 227.8 ± 13.8 mm. Hg produces complete saturation. This corresponds roughly to an oxygen concentration of 40%. The time required to reach complete saturation when the subject changes from breathing room air to 100% oxygen has also been determined—termed 'saturation time'. This, expressed as half-time, has been found to be 17.0 ± 1.7 seconds in normal subjects. In subjects with varying degress of pulmonary damage, caused by a number of diseases, significant differences in respect to both these measurements have been found. The results obtained so far correlate satisfactorily with independent clinical estimates of the subject's pulmonary disability. To find a known value at which the oximeter could be set with confidence, in every case blood-gas analysis was performed on a sample of arterialized-venous blood, taken while the subject was breathing pure oxygen, to ascertain that 100% oxygen produced complete saturation.

Tissue stimulators utilizing radio frequency coupling.
DONALD R. DUBBERT (introduced by OTTO H. SCHMITT). Univ. of Minnesota, Minneapolis.

The problem of isolating voltage stimuli used in tissue studies both conductively and capacitively from ground is discussed. A relatively simple method of achieving this isolation by means of radio frequency coupling is described in detail. The required stimulating voltage is used to amplitude modulate a radio frequency oscillator which is inductively coupled to a receiving circuit where the modulated carrier is reconverted into the original voltage stimulus by means of a small germanium crystal diode, operating as a linear rectifier, followed by a radio frequency filter. The resulting isolation is conductively complete, and the capacitance from output is of the order of only a few micro-microfarads. Certain design details which are of importance in obtaining satisfactory operation of the radio frequency stimulator as well as performance data are presented.

Effects of an antihistaminic in experimental shock.
H. E. EDERSTROM, M. R. DiGANGI AND CARL CALMAN (by invitation). Dept. of Physiology, St. Louis Univ., St. Louis, Mo.

Rats anesthetized with sodium pentobarbital were shocked by application of rubber bands to the hind legs for 3 hours and 40 minutes. Before release of the tourniquets 62 rats were given 20 mg/kg. Benadryl intraperitoneally. The 24-hour survival rate was 10% for the treated group, and 24% for 83 untreated controls. In dogs anesthetized with morphine and sodium pentobarbital burn shock was induced by immersion of hind limbs and lower abdomen in water at 90°C. for 5 seconds. Before immersion 16 animals were given i. m. 20 mg. Benadryl/kg, followed by 5 mg/kg. every 3 hours after the burn. The 24-hour survival rate in the treated group was 19%, and 25% in 16 untreated dogs. A second series of shocked dogs was treated initially with Benadryl as described above, but after the burn 40 mg. % Benadryl in saline was administered by intravenous drip at a rate permitting 100 cc/kg. to flow per 24 hours. Control animals received saline only at the same rate. In 11 Benadryl-treated animals survival was 36%, and in 11 controls was 27%. Blood pressure readings taken at hourly intervals in shocked dogs showed no significant differences between control and treated animals. Hematocrit readings taken every 3 hours suggested that the Benadryl-treated dogs did not show as great hemoconcentration as the controls.

Oximetry respiratory patterns in arterial hypoxemia with and without intra-cardiac shunt. JAMES O. ELAM, ALBERT ROOS AND J. F. NEVILLE, JR. (introduced by H. L. WHITE). Laboratory of Applied Thoracic Physiology (Surgery) and Dept. of Physiology, Washington Univ., St. Louis, Mo.

Fluctuations in the oximeter value as a result of the respiratory cycle have been studied in two groups of hypoxemic patients, the first group having no evidence of intra-cardiac shunt and the second group having interventricular septal defects demonstrable by cardiac catheterization studies. In the first group of hypoxemic patients the respiratory pattern in the oximeter value consisted of an increase in saturation 5–7 seconds after the beginning of inspiration followed by a decrease in saturation 5–7 seconds after the beginning of expiration. Such patterns were observed when the patient breathed room air at a rate of 4–6/min. and disappeared on breathing 100% oxygen. Apparently the slow respiratory rate permits depletion of increased alveolar pO_2 during expiration so that the following inspiration elevates the alveolar pO_2 and hence the relatively unsaturated arterial blood. The 5 to 7-sec. interval between the beginning of inspiration and the increase in the saturation agrees with the accepted values for lung-to-ear circulation time. The second group of hypoxemic patients who had intra-cardiac shunt showed a different respiratory pattern in the oximeter values with slow deep breathing. In this group the saturation decreased 2–3 seconds after the beginning of inspiration and increased 2–3 seconds after the beginning of expiration. Such patterns were elicited while the patient breathed room air or 100% oxygen. It appears that expiration caused a decrease in flow of the venous shunt blood relative to the oxygenated blood entering the aorta and that inspiration reverses this effect so that relatively more of the shunt blood enters the aorta. The 2 to 3-sec. interval between inspiration and the saturation increase suggests heart-to-ear circulation time. Occurrence of the pattern during breathing of 100% oxygen excludes the alveolar pO_2 role in this pattern as distinguished from the respiratory pattern obtained in patients without intra-cardiac shunt.

Cross-acclimatization to heat and cold. JOHAN W. ELIOT, HAROLD J. STEIN AND RICHARD A. BADER (introduced by H. S. BELDING). Quartermaster Climatic Research Laboratory, Lawrence, Mass.

Three healthy men were physically conditioned by walking 7 miles a day for 10 days, and were then exposed successively to 19 5-hour periods of heat (107°F.

dry bulb; 89°F. wet bulb; and 3 m.p.h. wind velocity), 14 5-hour periods of cold (−20°F., wind 3–4 m.p.h.), and 5 re-exposures to heat. After 5 weeks without environmental stress, 3 more heat exposures were given. The most striking finding of these studies was that, in men acclimatized to heat, loss of acclimatization was not accelerated by repeated cold exposures, as judged by cardiovascular and metabolic indices and subjective reactions. More rapid and effective vasoconstriction with successive cold exposures was demonstrated. Continuous diuresis and increased chloride loss were observed during the period of cold exposures, and these trends were only slowly reversed upon re-exposure to heat. No significant difference in basal metabolic rate was observed between the hot and cold periods. Blood, plasma and 'available fluid' volume studies revealed no consistent changes during the hot or cold periods, but plasma protein and hematocrit values showed definite hemoconcentration in the cold and hemodilution in the heat. It has not been demonstrated that increased tolerance to cold results from any of the changes observed.

Single fiber limulus nerve preparation for action potential analysis. LESTER ERICKSON (introduced by Otto H. SCHMITT). Univ. of Minnesota, Minneapolis.

Limulus leg nerves were studied as a possible source of single nerve fibers for dissection by the technique of Hodgkin. Histological examination showed the presence of numerous axons larger than 20 microns in a circumscribed area of the nerves studied. Histological data on these nerves was not found in the literature so fiber distribution studies were made similar to work done on squid by Young. Enlarged photomicrograph prints were used for these studies. The total number of fibers in Limulus leg nerves was estimated to be about 8000. Detailed fiber measurement and counts from the circumscribed area of the nerve containing the large axons gave an average of 435 fibers in the area. In the circumscribed area, 51% of the fibers were smaller than 5 microns; 21% of the fibers were from 5–10 microns; 18% were from 10–15 microns; 5% were from 15–20 microns in diameter and 5% were greater than 20 microns in diameter. When histological study showed that Limulus nerves might be a satisfactory source for single fiber preparations, dissection of the nerves was attempted. It was found possible to isolate the large diameter axons of 20 microns and make satisfactory single fiber preparations from such fibers. The technique and equipment was perfected so that preparations could be made for oscillographic analysis of action potentials propagated by single fibers.

Effect of vagotomy on intestinal motility. S. FAIK, F. C. MANN, AND J. H. GRINDLAY. Mayo Foundation, Rochester, Minn.

A study was made of intestinal activity, photographically recorded, in a group of trained dogs before and after transthoracic vagotomy. The apparatus was designed to record the slightest change of diameter in the intestine, and was attached to exteriorized loops prepared at different levels of the alimentary tract. In several instances 3 loops were prepared on the same dog, duodenal, jejunal and ileal. Various stimuli were applied and recordings were made. Emptying time of the stomach was delayed after vagotomy. This was shown by the necessity of fasting the dog for 48 hours in order to show complete quiescence in the loops in contrast to 26 hours fast in the nonvagotomized dog. Vagotomy abolished the intestinal activity caused by the slight and smell of food. The feeding reflex was delayed and of a shorter duration. Peristaltic waves were less in number, of shorter duration and occurred at longer intervals. This was especially marked in the ileum. The rhythmic waves were not affected in number or in their law of gradience.

Effect of diabetes and insulin on glucose Tm and other renal functions. SAUL J. FARBER, NEAL J. CONAN, JR., AND DAVID P. EARLE, JR. (introduced by HOMER W. SMITH). New York Univ., New York City.

The maximum tubular reabsorption of glucose (TmG) by the kidney was examined in 10 diabetics. Filtration rates were found to be within normal limits as were most of the absolute TmG values. However the GF/TmG ratios were lower than normal indicating a disproportion in glomerular and tubular function. Thus, the kidney tubule of the diabetic reabsorbs more glucose in proportion to the amount of glucose filtered. The action of insulin on the glucose Tm was examined in normals and diabetics. Insulin reduced the Tm in some of the normals examined and in all the diabetics. The GF/TmG ratio increased toward normal value in the diabetics in approximately the same proportion as the decrease in TmG. In several patients the excretion of glucose actually increased after insulin despite the fall in blood sugar. The effect of glucose and glucose plus insulin on the excretion of Na, K and PO_4 was examined in a limited number of diabetics. The excretion of K and PO_4 usually decreased as the blood level fell after the administration of glucose and glucose plus insulin. The excretion of Na increased after glucose. In a few instances the excretion of Na diminished after insulin despite the fact the excretion of glucose increased.

Secretion of insulin in the dog studied by means of the pancreatic-femoral anastomosis. PIERO P. FOA, JAY A. SMITH (by invitation), AND HARRIET R. WEINSTEIN (by invitation). Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago, Ill.

A disagreement exists regarding the nature of the hypoglycemic phase of the normal glucose tolerance curve. Some authors believe that it is due to stimulation of insulin production, while others attribute it to a protracted inhibition of liver glycogenolysis. The problem was investigated by means of 38 cross-circulation experiments on fasted heparinized dogs. The pancreato-duodenal or a branch of the mesenteric vein of the donor (A) was anastomosed with the femoral vein of the recipient (B). Return circulation was from the femoral artery of B to the femoral vein of A. After a control period, 5 cc/kg. of a 20% solution of glucose or the same volume of iso-osmotic (4%) saline was injected intravenously into A. Blood samples were taken for 75 minutes following the injection, the anastomoses were then disconnected and the sampling continued for 2 more hours. Blood sugar was determined according to Folin and Malmrose. The glycemia of a dog receiving pancreatic blood from a donor injected with glucose decreases sharply reaching a minimum in 30 to 60 minutes and gradually returning toward normal after the anastomosis has been disconnected. No changes are produced if saline was injected into a dog A. When the mesenteric vein of A was used, the glycemia of B increased instead of decreased. The hypoglycemia of B was of the same order of magnitude as that observed in the normal glucose tolerance curve, and appeared to be due to an increase of insulin content in the pancreatic blood of A. The results are consistent with the hypothesis that a rise in blood sugar concentration stimulates the secretion of insulin and that the pancreas plays a primary role in the production of the hypoglycemic phase of the normal glucose tolerance curve.

A rapid infrared gas analyzer. RICHARD C. FOWLER (introduced by W. O. FENN) Dept. of Physiology and Vital Economics, Univ. of Rochester, Rochester, N. Y.

A selective gas analyzer, suitable for operation with any gas having a unique infrared absorption band of measurable magnitude is described. These include CO_2, CO, ether, acetylene, nitrous oxide, and others. Its features at present include an instantaneous sample volume of approximately 0.5 cc. obtained by crossing the measuring beams, and an overall response time of 90% full sacle in 0.15 seconds obtained through the use of rapid 'SETT' bolometers. It is

adaptable for both static and continuous flow measurements. A calibration curve and sample results are shown.

Non-uniformity of lung ventilation. WARD S. FOWLER (introduced by JULIUS H. COMROE, JR.). Dept. of Physiology and Pharmacology, Univ. of Pennsylvania, Philadelphia.

Continuous analysis (Lilly-Hervey nitrogen meter) of N_2 content and volume flow of alveolar gas expired after one inspiration of 99.6% O_2 showed the N_2 content to increase several per cent as expiration continued. This indicates that *a*) inspired gas is not evenly distributed throughout the functional residual air and *b*) with quiet breathing the poorly ventilated areas of the lung empty proportionately more later in expiration. In 38 of 40 healthy men, increasing N_2 content was found in alveolar gas of a quiet tidal expiration. The magnitude of the variation in N_2 content has been expressed in terms of the relative dilution of alveolar N_2 by inspired O_2, and was affected by inspired volume, pre-inspiratory lung volume, inspiratory breathholding, expired volume and manner of expiration. When inspiration began at the maximal expiratory level, added dead space appeared to increase the N_2 variation. Decreased variability of N_2 content was found with voluntary and post-exercise hyperpnea.

Action potentials from single auditory nerve fibers. ROBERT GALAMBOS AND HALLOWELL DAVIS. Harvard University, Cambridge, Mass., and Central Institute for the Deaf, St. Louis, Mo.

We have previously reported on the electrical response of single units in the auditory nerve of cats: When a glass micropipette is introduced into the 'nerve', sharp spikes are elicited by acoustic stimuli. These spikes have been assumed to represent conducted impulses in auditory nerve fibers. For various reasons, it now seems more likely that they represent the discharge of the soma of second order cell bodies in the auditory tract. Some anatomical and physiological reasons for this conclusion will be presented.

Cortical projection of proprioception. J. R. GAY AND E. GELLHORN. Laboratory of Neurophysiology, Univ. of Minn., Minneapolis.

Little direct evidence has appeared in the literature on the effect of proprioceptive impulses in the cerebral cortex in animals and man. The proprioceptors were stimulated by passive movements of the extremities or stimulation of the peripheral end of ventral spinal nerve roots by condensor discharges. Recordings were made with an ink-writing crystograph from the exposed cerebral cortex in 36 cats and 8 monkeys under Dial-urethane anesthesia. It was found that stimulation of the proprioceptors by passive movements of the extremities or stimulation of the peripheral end of a ventral spinal root resulted in an excitation of the electrical potentials of the contralateral sensorimotor area in the cat and the contralateral precentral motor area in the monkey. The excitation tended to be more diffusely spread in the contralateral hemisphere in the cat. In the monkey the excitation was largely restricted to the contralateral precentral motor cortex. These experiments support the work of Bard and his associates and indicate that the cerebral cortex is involved in the proprioceptive system. It would appear that the cortical projection area of proprioceptive impulses is the sensorimotor area in the cat and the precentral motor cortex in the monkey.

Proprioception and the motor cortex. E. GELLHORN. Laboratory of Neurophysiology, University of Minn., Minneapolis.

The effect of proprioceptive impulses originating in muscles under conditions of temporary fixation of one or more joints was studied on the reactivity of the motor cortex to electric stimulation by means of EMG's. Results:

Fixation of a muscle at increased length (e.g., fixation of the elbow at an obtuse angle for the biceps) greatly increases the response of this muscle to stimulation if the proper cortical focus was chosen. This effect is largely due to impulses set up while the muscle develops tension isometrically. Proprioceptive impulses thus induced become also effective in those muscles with which the proprioceptively excited muscle forms specific functional associations. For instance, proprioception originating in the biceps increases also the reactivity of the extensor carpi to cortical stimulation; similar relations exist between triceps and flexor carpi, triceps and hamstrings, and hamstrings and anterior tibial muscle. It was shown also that this effect occurs in the opposite direction within these complexes. For example, fixation of the wrist in ventroflexion increases the reactivity to cortical stimulation not only of the extensor carpi but also of the biceps muscle. These effects seem to be based on the interaction of proprioceptive impulses with those elicited by cortical stimulation, resulting in an increased number of discharging motor units. This interpretation is suggested by the decreased summation time, the greater and more rapidly increasing amplitude of the EMGs and occasionally, by the increased after-discharge.

The membrane potential of single muscle fibers. R. W. GERARD AND G. LING (by invitation). Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

We have previously reported (*Federation Proc.*, *1948*, and in press) that all surface fibers of the frog's sartorius normally have a membrane potential (measured with a capillary microelectrode) within a couple of millivolts of 80. This value is insensitive to changes in pH, calcium, phosphate, or length, but varies with potassium, stimulation, and disturbed metabolism. An A fraction, above $55m^B$, is directly dependent on metabolism (CrP concentration); a B fraction, below $55 m^B$, is only indirectly so. With both glycolysis and respiration blocked (IAA $\pm$ CN), the potential falls to zero in 2 to 4 hours at room temperature. Two points are emphasized here. 1) Muscle fibers may be in full rigor (isotonic $CaCl_2$) yet exhibit a normal membrane potential. Conversely, fibers may have a potential under 20% normal (caffeine, high KCl and $CaCl_2$) and yet give seemingly normal contractions. There is no necessary parallelism between relaxation, contraction, or contracture and the size of the membrane potential. 2) Stretch does not alter the membrane potential, but is known to increase the resting metabolism (Feng). Since a muscle fiber may be normal at various lengths and since its surface area must vary as length $\frac{1}{2}$, it seemed possible that the extra metabolism of stretched muscle was required to maintain the extra area of membrane fully polarized. If so, when metabolic energy is blocked (IAA + CN), a stretched muscle should depolarize faster than its unstretched partner. This is the case. Preliminary results indicate that the rate of depolarization may by proportional to total fiber surface; which would suggest that much of the resting metabolism is used to maintain membrane integrity and polarization.

Effect of microwave diathermy on the peripheral circulation and on tissue temperature in man. J. W. GERSTEN, K. G. WAKIM, J. F. HERRICK AND F. H. KRUSEN. Mayo Clinic and Foundation, Rochester, Minn.

The effect of microwaves at a frequency of 2450 megacycles/sec. on the peripheral circulation and on the tissue temperature was studied in 50 normal subjects. Two hundred fifty four observations were made, with the output of the microwave generator either 60 or 80 watts, and with the duration varying from 1 to 30 minutes. The hemispherical director with a diameter of 9.5 cm. was used at a distance of 5 cm. from the exposed surface. The blood flow in the forearms was determined by the use of the plethysmograph with the compensating spirometer recorder, and the skin, subcutaneous and muscle temperatures were recorded by means of thermocouples. After control blood flow and temperatures were established the forearm was exposed to microwaves, and blood flow and temperature studies were again made when the microwaves were turned off. Significant increases in blood flow and in tissue temperature were produced in the extremity exposed to microwaves, but the changes in body temperature, heart rate, and blood flow of the unexposed extremity were insignificant. The average rise in muscle temperature was significantly greater than that of subcutaneous tissue, while the average increase in subcutaneous temperature was greater than that of the skin. The average increases in temperature were highest after 20 minutes of irradiation, and were practically the same for 60 and 80 watts. After 20 minutes irradiation with 80-watt output, the temperature rise was 6.7°C. in the muscle, 5.8°C. in the subcutaneous tissue, and 4.7°C. cutaneous. With heating prolonged to 30 minutes, the tissue temperature decreased significantly from the level reached at 20 minutes, with a greater decrease after 80-watt than after 60-watt output. The average blood flow increase in the exposed extremity after 30 minutes of heating was 65%. An S-shaped curve resulted when blood flow increase after exposure at 80 watts was plotted against duration of exposure. After 30 minutes of heating the decline in tissue temperature from the peak attained at 20 minutes was proportional to the increase in blood flow.

Changes in response and in excitability of the perfused turtle ventricle with 'fatigue' and with strophanthin. A. S. GILSON, JR. Dept. of Physiology, Washington Univ., St. Louis, Mo.

Experiments have been conducted with the turtle ventricle using a Straub cannula and ligation between atria and ventricle. With bicarbonate buffered oxygenated solutions, addition of strophanthin gave no improvement of mechanical response. Inconstantly, the drug caused prolongation of the measured relatively refractory period but this occurred during a brief period of increasing threshold leading to frank intraventricular blocks. Phosphate buffered solutions yielded results in this latter respect of generally similar nature. A few preparations perfused with phosphate buffered solution for times approaching 30 hours were driven at rates approximately 30 per minute except for brief periods of testing. Driving at more rapid rates, early in the experiment, shortened the R-T interval progressively to a value slightly below normal for the rate used and there was slight shortening of R-T below normal, though only for a few beats, upon return to slower driving rate. With progressive mechanical failure there was progressive shortening of R-T (and of mechanical systole) the change being slight for short cycle lengths, great for long cycles. It was not possible to measure consistent increases of relative refractory

phase adequate to account for this on a basis of the assumption that shortening of the duration of systole measures relative refractoriness. Thus, in the development of the hypodynamic state by this means, minimum duration of systole is but slightly shortened, maximum duration is greatly shortened. This condition is apparently not necessarily or directly related to changes in the duration of the measured relative refractory phase.

Olfactory acuity and the sensation complex of appetite and satiety: The influence upon olfactory acuity of defatted, dehydrated duodenum of hog (viobin). MARGARET GOLDSCHMIDT (by invitation), PHILLIP J. RAIMONDI (by invitation) AND FRANZ R. GOETZL. Dept. of Medical Research, Permanente Foundation, Oakland, Calif.

Olfactory thresholds (Elsberg's method) were determined in normal individuals daily at regular intervals. On test days the subjects' statements regarding appetite and their caloric intake were recorded. Meals were found to be preceded by a period of increasing and followed by one of decreasing olfactory acuity. The precibal increase in olfactory acuity could be prevented by intercibal ingestion of food, the decrease in that acuity by omission of meals. It is suggested that by estimating diurnal variations in olfactory acuity, measures may be found for the sensations of appetite and satiety. Benzedrine was observed to produce decrease in olfactory acuity, decrease in the intensity of the sensation of appetite, decrease in caloric value of freely selected meals and, also, a sensation of satiety. It was felt that the conversion of the sensation of appetite into one of satiety as normally follows ingestion of food may depend upon results of interaction between certain constituents of food with certain components of the gastro-intestinal tract. Such interaction might lead to formation or release of substances directly responsible for the change in sensation. Thinking along these lines, it appeared interesting to investigate possible influences upon the sensation complex of appetite and satiety, upon olfactory acuity, and upon caloric value of freely selected meals of defatted, dehydrated but otherwise unaltered duodenum of hog (VioBin). In the experiments presently reported, it was found that in normal human subjects defatted, dehydrated duodenum of hog upon oral ingestion is capable of decreasing olfactory acuity, of depressing the sensation of appetite, of creating a sensation of satiety and probably also of diminishing food intake. Structure and occurrence in other tissues of the constituent of the duodenal preparation responsible for the effects described are being subjected to further investigation.

Myocardial lactate and pyruvate metabolism in normal intact dogs, as studied by coronary sinus catheterization. WALTER T. GOODALE, DONALD B. HACKEL, MARTIN LUBIN AND PAULINE P. WILSON. Medical Div., Army Chemical Center, Md.

Myocardial utilization of lactate and pyruvate has been demonstrated by several investigators in isolated heart, heart-lung, and open chest experiments. Development in this laboratory of a technique of catheterizing the coronary sinus under fluoroscopic control has made it possible to study myocardial metabolism in normal intact dogs, unanesthetized as well as under light nembutal anesthesia. Measuring coronary A-V differences, and coronary blood flows by the nitrous oxide method, lactate and pyruvate utilization was found to be even greater than in less physiological preparations. The total lactate and pyruvate uptake accounted for 20–70% of the total myocardial uptake of oxygen. Of greatest interest was the similar linear relationship between the utilization of these two metabolites and their arterial concentrations, whereas no such relationship was found for glucose. Coronary A-V glucose differences were usually low, with such a high sampling error as to be statistically insignificant. Lactate and pyruvate appear to be preferred sources of energy for the myocardium, and are increasingly utilized as their arterial levels rise. This fact may provide a logical explanation for the excellent adaptation of the heart to stress, as to the increased work of exercise, during which lactate and pyruvate tend to accumulate in the blood from contraction of the skeletal muscles.

Influence of emotions and feeling states on the behavior of the human colon. WILLIAM J. GRACE, STEWART G. WOLF AND HAROLD G. WOLFF. With the technical assistance of CATHERINE R. LEE AND PAUL SETON. Depts. of Medicine and Psychiatry of the New York Hospital and Cornell Univ. Medical College, New York City.

Previously attempts at studying the function and behavior of the human colon have been made by using x-rays, motor meals, balloon kymography, inspection of the colon through sigmoidoscopy, and inspection of the activity of exposed loops of bowel. The viewpoint of most of the studies has been pharmacologic and descriptive. We have had a unique opportunity to study the behavior of the human colon in two fistulous subjects with particular emphasis of the influence of emotions and feeling states. Our findings indicate that situations productive of anger, guilt, resentment, and hostility are accompanied by hyperfunction of the large bowel. This hyperfunction is manifested by an increase in motor activity, blood flow lysozyme production, and usually an increase in mucus secretion.

In life situation productive of intense fear and fright there occurred a pallor, and relaxation of the large intestine. Increase in motor activity, blood flow and secretion of the large bowel occurred regularly following the ingestion of an average meal. However in one of our subjects little change in activity was noted when he was in a period of low spirits, dejection and mild depression. Other threats to bodily and personal integrity such as sigmoidoscopic examinations, personality study and having the patient perform a psychometric test resulted in an increase in motor activity and blood flow. A period of sustained anger, resentment and hostility resulted in a profuse eruption of petechiae throughout the surface of the colon.

Effect of bile on gastric mucus. RHODA GRANT (by invitation), M. I. GROSSMAN AND A. C. IVY. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

The effect of bile on mucus secreted under acute conditions by the cat's gastric mucosa after exposure to 1% acetic acid has been studied. Mucus collected under these conditions is opaque, white, extremely viscous, with a *p*H of 7–8.5 except occasionally towards the end of the secretion period when it falls to 2 to 3. The change in reaction does not change its gross appearance. Microscopically, however, intact gastric surface cells are suspended in the alkaline; the nuclei alone in the acid state. Bile or bile salts formed with the mucus, at room temperature and at an optimum *p*H in the range 7 to 8.8, a transparent homogeneous mixture. Its viscosity, in contrast to that of the original mucus, could be measured by the Ostwald method. When mixed with saline, clear acid gastric juice, 0.2m Na₂H PO₄ or duodenal pouch juice the mucus remained in a separate phase even in the optimum *p*H range. The cellular elements disappeared from the mixture. Opacity and high viscosity of the original mucus was due apparently to the cellular elements suspended in it. Disintegrative changes in the gastric surface cells have been found in mucosa exposed to bile for a few minutes—the extent of this depending on the state of maturity of the cells—consequently on the amount of mucus they contained. Cytolysis of these cells by bile suggests potential danger to the mucosa should suitable conditions for the action exist.

Influence of environmental temperature on the metabolic response to injected pyrogens. RONALD GRANT (introduced by VICTOR E. HALL). Dept. of Physiology, Stanford Univ., Calif.

Rabbits given typhoid-paratyphoid vaccine (0.01 ml/kg. of vaccine containing 2 × 10⁹ organisms per ml.) show a biphasic temperature rise, beginning when heat defence mechanisms become inhibited, about 15 minutes after injection. Interruption of the temperature rise due to restored activity of heat defence mechanisms occurs in the second hour. Renewed inhibition causes the second rise. At 0°C. the fever curve is similar but with less rise. Shorn animals exposed at 0°C. maintain constant rectal temperatures with strong shivering, oxygen consumption being increased 120 per cent. Given vaccine, they may show slight fever in the first hour but usually develop decided hypothermia in the second hour with obvious reduction of shivering. Fall of temperature may be checked in the third hour by restoration of shivering. Oxygen consumption is slightly increased during the first hour (mean increase 1.76 ml/kg/min., or 17% of the control value at 20°C.). This increase is maximal 20 to 30 minutes after injection. Magnitude of the increase is unaffected by environmental temperatures within the limits of heat and cold tolerance. In the second and subsequent hours at moderate and high temperatures oxygen consumption remains elevated largely because of the van't Hoff effect. At low temperatures there is decided inhibition of oxygen consumption in the second hour, especially in shorn animals, with recovery, usually, in the third hour. Closely similar results were obtained using 'Pyrexin' (an endogenous pyrogen prepared by Menkin from aseptic pleural exudate).

Aerobic glycolysis in the anesthetized dog. MELVIN GRAY (by invitation), LOWELL E. HOKIN (by invitation) AND WARREN S. REHM. Dept. of Physiology, Univ. of Louisville, Louisville, Ky.

Lutwak-Mann (*Biochem. J.* 41: 19, 1947) reported aerobic glycolysis in the presence of added glucose in the rat's stomach. The present work is concerned with the rate of glucose consumption and lactate production in the secreting stomach of the anesthetized, heparinized dog. A portion of the stomach with an intact blood supply was placed in a chamber. The vein from the stomach in the chamber was cannulated and the total outflow was collected. Arterial blood was collected simultaneously. The volume of secretion and the quantity of HCl secreted were determined. The glucose consumption and lactate production were calculated from the A-V difference, volume of secretion, and blood flow. The Shaffer-Hartmann-Somogyi method was used for glucose and the microdiffusion method of Winnick for lactate. Glycogen determinations were made on samples of stomach by the method of Good, Kramer, and Somogyi, both before and after the experimental periods. In five out of six dogs the decrease in glycogen was less than 10% of the glucose consumption. The average lactate production was 25% of the glucose consumption. The rate of HCl

production was roughly proportional to glucose consumption. These data disprove the Bull-Gray theory (*Gastroenterology* 4: 175, 1945) of HCl formation because much more HCl is secreted than could originate from glucose going to lactic or pyruvic acid (see Davies *et al.*, *Nature* 159: 468, 1947).

Gastroduodenal ulceration in dogs produced by continuous intragastric administration of HCl-pepsin solutions. M. I. GROSSMAN AND M. J. FOGELMAN (by invitation). Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

In previous experiments we had shown that continuous intragastric infusion of o.1 N HCl at a rate of 10 cc/kg/hr. for 72 hours produced gastro-duodenal ulceration in all dogs and this effect could be prevented by simultaneous continuous intravenous infusion of isotonic NaHCO₃ at a rate sufficient to maintain normal blood *p*H. In the present experiments three groups of dogs were used. *Group I*, consisting of five animals, received o.10 N HCl with o.2% pepsin intragastrically; *Group II*, 14 dogs, received o.10 N HCl-pepsin intragastrically plus isotonic NaHCO₃ intravenously in amounts sufficient to maintain normal blood *p*H; *Group III*, 14 dogs, received o.15 N HCl-pepsin plus NaHCO₃ intravenously to maintain normal blood *p*H. *Group I* dogs all developed severe acidosis (blood *p*H 6.9–7.2) and gastroduodenal ulceration within three days. None of the dogs in *Group II* developed gastroduodenal ulceration even when the experiment was continued up to 10 days. All dogs in *Group III* which were kept going for 6 days or longer developed gastroduodenal ulcers (7 of 14 dogs). Under the circumstances of these experiments the threshold of acid concentration for ulcer production is between o.10 N and o.15 N.

Distribution of the phosphatides in rat-liver nuclei and cytoplasmic particulates. M. H. HACK (introduced by HUBERT R. CATCHPOLE). Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago.

Female white adult rats, maintained on an *ad libitum* diet of Purina Fox Checkers and water, were fasted 12–18 hours, killed by a blow on the head and the nuclei and cytoplasmic particulates isolated from pooled livers according to the procedures of Dounce (*J. Biol. Chem.* 147: 685, 1943) and Claude (*J. Exp. Med.* 84: 51–89, 1946) respectively. The phosphatide composition of the lyophilized nuclei, large-granules, microsomes and whole liver was determined by the method previously described (*J. Biol. Chem.* 169: 137, 1947). The results showed that as rats increased in weight (age) from 140 gm.–350 gm. the total phosphatide increased about 30% with the cephalin to lecithin ratio changing from 1:1–1:2. Assays run on

benzene extracts of the same tissues indicated that nearly half of the liver phosphatide was bound as a phosphatide-protein complex, mostly as lecithoprotein. Contrary to the observations of Stoneburg (*J. Biol. Chem.* 129: 189, 1939) and of Williams *et al.* (*J. Biol. Chem.* 160: 227, 1945) we have found nuclei to be the phosphatide-poor component of the cell. Our most homogeneous preparations showed the cephalin: lecithin ratio to be about 2:1, acetalphosphatide was absent and assays on benzene extracts indicated nearly 100% phosphatide-protein. The large-granule fraction seems to reflect most the liver phosphatide increase with growth which was due primarily to an increasing concentration of lecithin so that with increasing age the cephalin: lecithin ratio goes from 2:1 to 1:1. Acetalphosphatide was present and benzene extracts indicate that ⅓ to ½ of the phosphatide was bound to protein, largely as lecithoprotein. The phosphatide composition of the microsome fraction was found to remain quite constant with growth (140–350 gm. rats) with a cephalin: lecithin ratio of 1:1.4–1.6. About ⅓ was phosphatide-protein mostly of the lecithoprotein type. Acetalphosphatide was present in about the same concentration as in large-granules. However, there was present an unidentified 'acetal' which yielded a fuchsin addition product insoluble in capryl alcohol. Sphingomyelin was found to be either absent or of small concentration in whole liver and in the three cell fractions. The table shows the phosphatide distribution in the liver of 350 gm. rats. The data is expressed in uM/gm. dry weight of tissue.

	TOTAL PHOSPHATIDE	CEPHALIN	LECITHIN	SPHINGO-MYELIN
Whole liver.......	167.5	54.1	112.2	1.2
Nuclei...........	35.5	23.7	11.8	0
Large-granules....	229.2	107.3	112.7	9.2
Microsomes.......	321.0	129.2	191.8	0

Effects of drugs and physical measures on spastic skeletal muscle. NORMA M. HAJEK (by invitation) AND H. M. HINES. Dept. of Physiology, State University of Iowa, Iowa City.

A state of prolonged shortening or spasticity was produced in the gastrocnemius muscles of albino rats by the local injection of tetanus toxin. This condition was accompanied by atrophy and loss of strength in the affected muscles. Although temporary relaxation was elicited by curare injection, its frequent administration did not lessen the extent of atrophy and strength loss which occurred in spastic muscles. Treatment of shortened muscles by stretching reduced the amount of atrophy and strength loss. Curare facilitation did not enhance the beneficial effects of stretching. The results suggest that stretching spastic muscle without

curare is more beneficial. Excessive or 'over' stretching was found to be injurious to both normal and spastic muscles. Electrical stimulation of the spastic muscle in the fully stretched position resulted in less atrophy but greater strength loss per unit mass of muscle. Stimulation in the unstretched position proved to be ineffective.

Some recent observations upon physiologic action of anti-histamine drugs. B. N. HALPERN. French National Center of Scientific Research, Hôpital-La Charité, Paris, France.

Quantitative studies on capillary permeability have been made by following the passage of intravenously injected dye into the peritoneal cavity and into the anterior chamber of the eye. It has been shown that the antihistamine substance dimethylamino-2 propyl-L phenothiazine (phenergan) prevents the changes in rate of passage of the dye into the peritoneal cavity after peritoneal injection of histamine and that of fluoriscein into anterior chamber after intravenous histamine. It has also been shown that this pheneoskiazine derivative prevents acute pulmonary edema in rabbits following intravenous epinephrine or chloropicrite. It is concluded that this antihistamine substance is a potent agent in preventing increase in capillary permeability produced by histamine and certain toxics.

Effects of dihydroergocornine on the peripheral circulation in man. D. W. HAYES, K. G. WAKIM, B. T. HORTON AND G. A. PETERS. Mayo Clinic and Foundation, Rochester, Minn.

The effects of the intravenous administration of dihydroergocornine (DHO-180), an alkaloid of ergot, on skin temperatures, blood pressure, heart rate and peripheral blood flow were studied in 20 human volunteers. The action of the drug is chiefly sympatholytic and, therefore, the drug is considered to be a vasodilator. It was administered to 6 patients by intravenous infusion in a solution containing 0.5 mg. of dihydroergocornine per 100 cc. of physiologic saline solution and to 14 patients by a single intravenous injection. The total dosage varied from 0.25–0.4 mg. Control values for skin temperatures, blood pressure, heart rate and blood flow were determined before the drug was given, and the observations were again recorded at regular intervals for a period averaging 65 minutes after administration of the drug. The blood flow was determined by means of a venous occlusion plethysmograph with a compensating spirometer recorder. Skin temperatures were recorded by means of thermocouples applied to the skin over the forehead, over the right and left deltoid muscles and over the right and left quadriceps femoris muscles.

Dihydroergocornine produced an over-all average increase in peripheral blood flow of 95% in the upper extremities, and 68% in the lower extremities, in 19 of 20 cases. In spite of the increase in blood flow in the extremities the skin temperatures were slightly decreased, even during the maximal increase in blood flow. The blood pressure fell in the 2 hypertensive cases after administration of dihydroergocornine. The decrease of systolic pressure was 58 mm. Hg in one case and 30 mm. Hg in the other, while the diastolic pressure fell 18 mm. in the former and 10 mm. of mercury in the latter. In normotensive subjects there was no significant change in blood pressure. The heart rate decreased in every case, with an average reduction of 13 beats per minute. Side reactions were more frequent than had been reported by other investigators, even with lower dosage. Nasal congestion, nausea, headache, flushing, an urgency for urination and vomiting were the side reactions observed.

Rectal temperature patterns of dogs during peripheral vasodilation and vasoconstriction induced by the immersion method. ALLAN HEMINGWAY. Dept. of Physiology, Univ. of Minnesota, Minneapolis.

Rectal and brain temperature measurements of normal unanesthetized trained dogs were measured during a testing process which consisted of local application of heat and cold sufficient to induce thermal cutaneous vasomotor activity. The animals were suspended in a hammock in a room at 15–17°C. and after a 30- to 45-minute rest the hind legs were immersed in water at 44 to 45°C. which was followed by immersion of the same limbs in cold water. Thermal cutaneous vasoconstriction and vasodilatation were indicated by changes in the skin temperature of the ear. A variety of rectal temperature patterns produced during this test was obtained. In some instances immersion of the hind limbs caused a sharp fall in rectal temperature when cutaneous vasodilation occurred, an effect which cannot be explained by the theory of the central control of body temperature. In other instances a rising rectal temperature occurred with vasodilatation, this being in accordance with the classical 'thermostat' central control theory.

Cytology of the pituitary gland in experimental goiter. GEORGE C. HENEGAR AND GEORGE M. HIGGINS. Mayo Foundation, Rochester, Minn.

With the discovery of the goitrogenic effects of certain thiourea and sulfone derivatives, study has been given to the influence these drugs exert upon the physiology of the thyroid. Although changes in the cytology of the anterior lobe of the pituitary exerted by thyroidectomy are well known, cytologic changes in the pituitary of animals made goitrous by these drugs

are less clearly understood. Studies were reported of the percentage distribution of cells comprising the anterior lobe of young male rats which had received certain well known goitrogens, promizole, thiouracil and thiobarbital. Correlations between the cytology of the pituitary, the degree of hyperplasia of the thyroid, and the basal metabolic rates were presented. The percentage of basophils in the anterior lobe of goitrous rats is greatly increased; the percentage of acidophils is decreased and large hyaline bodies, resembling those seen in the so-called 'thyroidectomy' cells are observed. Responses of thyroid and of anterior lobe to these three goitrogens were identical in kind but variable in degree. Thyroids were greatly increased in size; acinar cell heights were greater, and there was a marked decrease in colloid. Oxygen consumption was reduced. The pituitary-thyroid relationships were discussed.

Cerebellar projections to the cerebral cortex in cat and monkey. ELWOOD HENNEMAN, PAULINE COOKE AND RAY S. SNIDER (introduced by H. W. MAGOUN). Dept. of Anatomy, Northwestern Univ. School of Medicine, and Illinois Neuropsychiatric Institute, Dept. of Psychiatry, Chicago.

In 20 cats and 6 monkeys (chloralosan or barbiturate anaesthesia) single shocks were delivered to the cerebellum with electrodes applied to its cortex or placed stereotaxically in its nuclei. Amplified potentials, led from the cerebral cortex with bipolar electrodes, were photographed from a cathode ray oscilloscope. Depth of anaesthesia and physiological status were crucial. Excitation of anterior lobe in cat and monkey evoked surface positive monophasic potentials (10 sigma latency) in contralateral cerebral motor and sensory areas. Paramedian lobules projected bilaterally to sensory-motor *areas I* and *II* in cat. Both cerebellar auditory areas in cat projected bilaterally to cerebral auditory regions, responses centering around tip of anterior ectosylvian fissure (Garol's motor pinna area) and adjoining suprasylvian gyrus (Woolsey's motor-ear area). Elsewhere in the auditory cortex responses were inconstant. In some experiments on both species excitation of cerebellar visual cortex elicited cerebral visual responses (20–25 sigma latency). Nuclear stimulations evoked cerebral responses not confined within single functional areas. All sensory-motor and auditory areas responded in both species. In monkeys areas 6 and 8 responded discretely. In cats, but not in monkeys, the visual areas responded frequently. Sensory-motor, visual, and cingulate projections were distinguishable on the medial aspect of the cats' cerebrum. Thus, all investigated cortical areas having motor function receive cerebellar projections.

Factors affecting the composition of alveolar air. F. A. HITCHCOCK AND R. W. STACY (by invitation). Laboratory of Aviation Physiology, Ohio State Univ., Columbus.

The mass spectrograph developed in this laboratory for continuously recording the partial pressures of O_2 and CO_2 during the respiratory cycle makes possible the study of the dynamics of respiration. Curves obtained in normal respiration and during maximal exhalations have been analyzed. These curves show that a slight drop in the pO_2 occurs before any change takes place in the pCO_2. A fraction of a second after this initial change there is a rapid drop in the pO_2 and a still more rapid rise in the pCO_2, which accounts for almost the entire difference between inspired air and alveolar air. Following this there is a slow increase in the pCO_2 and a slow decrease of pO_2, which continues until the flow of air out of the respiratory tract ceases. It appears that an important factor determining the partial pressure of alveolar air obtained by the Haldane-Priestly technique is the duration of the expulsion. During exhalation the pCO_2 increases while the pO_2 decreases at rates which may be as great as 2 or 3 mm. per second. Respiratory quotients calculated at $\frac{1}{2}$-second intervals during maximal exhalation show that the quotient during the first second is high, invariably being above 1. The value of the quotient is rapidly reduced and continues to drop as long as exhalation continues.

Adequate cortical stimuli in the production of autonomic responses. E. C. HOFF, A. C. JOHNSON, D. M. SHOLES AND E. H. GRAY. Medical College of Virginia, Richmond.

An experimental investigation of the characteristics of chemical and electrical stimuli which may evoke autonomic responses from the cerebral cortex has been carried out in the cat. Acetyl β-methylocholine chloride (Mecholyl, Merck) in saline solution, applied topically to the cortex produces profound falls in blood pressure with latencies within 15 sec. The threshold concentration for this response is lower, with shorter latency and greater magnitude, in the electrical pressor area of the cortex than in the electrical depressor area. Prior application of di-isopropyl fluorophosphate (DFP) to a cortical focus greatly lowers the threshold for the Mecholyl effect. In a typical experiment, DFP reduced the threshold concentration of Mecholyl from 2.5 mg./cc. to 0.3125 mg./cc. Topical application to the cortex of other cholinesters, including acetylcholine chloride, carbamylcholine chloride (Carcholin, Merck) and urethane of β-methylcholine chloride (Urecholin, Merck) likewise evoked sharp falls in blood pressure; but with these compounds no threshold-lowering effect of DFP could be demonstrated. Using

an electronic square-wave generator to deliver stimuli to the cortex, it was found that the threshold potential for autonomic responses is quite constant at 3 to 4 volts, the optimum range being reached just above threshold potential. Below threshold potential, no alterations of frequency or pulse duration produced a response. Frequencies of 30 to 180 cycles per sec. are within the optimum range and the extreme range is between 5 and 500 cycles. Pulse durations of 4 to 8 millisec. apparently represent an optimum at a frequency of 60 cycles.

Regeneration of nerve fibers to sweat glands. W. HENRY HOLLINSHEAD, Section on Anatomy, Mayo Foundation and Clinic, Rochester, Minn.

Anomalous patches of sweating appearing in areas denervated by sympathetic chain ganglionectomy may be due either to failure to interrupt all the fibers supplying these areas or to subsequent regeneration. Since preganglionic fibers regenerate readily, and are cholinergic as are the postganglionic fibers to sweat glands, it is conceivable that sweat glands can be reinnervated by regeneration of preganglionic fibers. In order to test this possibility, the original cephalic end of the cervical sympathetic chain, after removal of the superior cervical ganglion, was anastomosed in the arm to either the median or the ulnar nerve. The flexor nerve not used for anastomosis was resected from the arm, but the radial nerve was left intact. The high skin resistances typical of the operated side began to decrease after the fourth month, and sweating thereafter reappeared on the previously denervated and dry foot pads. Section of the anastomosed nerve after sweating was well established failed to alter either sweating or skin resistance; subsequent section of the radial nerve again denervated the sweat glands. Thus functional regeneration was not due to regeneration of the anastomosed preganglionic fibers, but rather to growth from the intact radial nerve. Histologic examination of the anastomosed nerves indicated that the fibers of the sympathetic chain had entered the degenerated nerve in great numbers, but had failed to grow far enough along this to reach the level of the wrist, even though allowed ample time to do so.

Effects of stimulation of the motor cortex after deafferentation. J. HYDE (by invitation) AND E. GELLHORN. Laboratory of Neurophysiology, Univ. of Minnesota, Minneapolis.

Gellhorn (1948) observed the increased electromyographic EMG response to stimulation of the motor cortex which can be induced by fixating a joint to favor a muscle proprioceptively. The magnitude of this proprioceptive facilitation prompted an investigation to study the relative importance in the EMG response

of motor cortex and spinal cord. Muscle responses to cortical stimulation were studied before and after section of lumbo-sacral posterior roots. In 'Dial' cats the motor cortex was stimulated with condenser discharges. EMGs were recorded with an Offner crystograph through copper wires sewn into semi-tendinosus and tibialis anticus. The latter muscle was tenotomized and its isometric tension recorded at different initial lengths. The limb was fixed, thus preventing lengthening of any intact muscles. Results obtained thus far indicate that slight variations in the frequency or intensity of motor cortex stimulation are capable of causing appreciable differences in the amplitude of EMG of muscles at a given initial length even after deafferentation has removed the possibility of proprioceptive recruitment. Further, confirmation of the idea that the EMG is dependent on the number of active motor units, whereas the tension developed is not (see Loofborrow, 1948) was obtained under new conditions: in the control experiments, an increase in the initial length of tibialis resulted in an increased EMG as well as an increased tension in response to a given cortical stimulus; after deafferentation, however, while the tension increased with an increased initial length (as would be expected from the tension-length diagram), the EMG was unchanged by alterations in initial length of the muscle.

Fluid shifts in animals during pressure breathing. CHESTER HYMAN AND JOSEPH GOODMAN. Dept. of Physiology, Univ. of Southern California, Los Angeles.

A satisfactory system for subjecting anesthetized animals to automatically controlled high pressure artificial-pressurized respiration has been developed. The system consists of a controlled source of pressure for inspiration and a similarly controlled loading against the expiratory pressure. The rate of respiration is determined by a telechron-operated switch which alternately opens inspiratory or expiratory solenoid valves. The magnitude of the pressures employed necessitates the use of proper protective counter-pressurization for the animals in order to prevent severe damage to the lungs. This protection is afforded by a system consisting of an air-tight bladder fitted to the animal and held in place by a non-elastic cloth jacket. A suitable pressure-tight helmet has been constructed to permit pressure breathing at these high levels without requiring direct tracheal cannulation. These garments and helmet are built to "pressurize" the entire thoracic and abdominal surfaces of the animal, but in no way protect the four limbs. Fluid loss from the circulation of animals exposed to pressure breathing under these conditions was determined by the degree of concentra-

tion of several blood constituents. One cc. samples were obtained from the exposed femoral arteries for the determination of hematocrit, hemoglobin, and plasma protein values. In addition, the concentration of previously injected non-diffusible dyes was determined. The general findings indicate a marked hemoconcentration, paralleled by a rise in the hemoglobin and plasma protein values. The fluid loss so determined was variable, but at least partially dependent on the level of pressure breathing maintained. The condition of the animal at the time of the experiment seems to play a major role in determining the final fluid loss. The fluid loss may be considered as a filtration of fluid from the abnormally high pressure in the capillaries into the more nearly normal pressures existing in the unprotected tissues of the limbs.

Some observations on electromyography. V. T. INMAN (by invitation), B. FEINSTEIN (by invitation) AND H. J. RALSTON. Univ. of California Medical School and College of Physicians and Surgeons, San Francisco.

Electromyographic studies of human voluntary muscle in cineplastic and normal subjects reveal the absence of measurable electrical activity in most muscles during rest, and even in various muscles of the lower extremity during certain phases of locomotion. The prevailing conception of muscle tone is questioned. Due to the existence of the length-tension diagram, there is no regular relationship between EMG amplitude and isometric tension, except at a given length of muscle. The use of electromyography in the study of phasic action of muscle groups is briefly discussed. The abrupt cessation of electrical activity in antagonists upon contraction of agonists is noted. It is shown that the amplitude of the EMG diminishes in the intact muscle as muscle length increases. This finding is in disagreement with the studies of Loofbourrow (*J. Neurophysiol.* 11: 153, 1948) on cortical stimulation of cats. Experiments on frog muscle-nerve preparations also do not agree with the findings of Loofbourrow on cat muscle, EMG amplitude increasing when the muscle stretched. The work of Duyff and Wiggers (*Arch. Neerl. Physiol.* 27: 195, 1943), showing decrease in chronaxie of isolated curarized, but not of isolated normal frog muscle under stretch, and decrease in chronaxie of intact muscle under stretch, is discussed in these connections.

Theory of essential hypertension in man. EDMUND JACOBSON. Laboratory for Clinical Physiology, Chicago, Ill.

As previously reported (A.A.A.S.), early essential hypertensive disease in man apparently can often be arrested by persistent application of progressive relaxation methods. Advanced cases commonly show persistently lowered pressure with lessened symptoms. Man differs from the experimental animal in that hypertensive disease arises in him during the efforts of struggle for existence. Efforts differ in races and cultures with the pace of living; if essential hypertension is a cultural disorder, medications, hormones, antirennin and other products may abate the symptoms yet fail to solve the basic problem. Muscle is the tissue by which man moves and imparts force to his environment. All effort, including so-called mental effort, proceeds by shortening of muscular fibers. Neuromuscular relaxation is freedom from effort, physical and mental. In relaxed muscle, most capillaries are closed. Upon contraction, they open in increasing numbers, due to action of metabolites. Increased blood pressure increases supplies and removes contraction products. Muscular contraction raises blood pressure through complex nervous reflexes and action of hormones and probably of metabolites. This effect often is masked. The glandular system possibly participates through nervous stimulation or through hormone action. The rôle of the kidney is still undetermined. Upon marked skeletal muscular contraction, renal arteriolar contraction (like retinal) increases, often to spasm. Renal anemia may increase production of angiotomin. Perhaps blood is shunted from the cortex (Truetta). Thus in early hypertension, arteriolar sclerosis is often absent (Smithwick). Arteriosclerosis perhaps results from wear and tear especially from excessive effort. Individuals differ in hereditary susceptibility (Ayman). On this theory, the failure to reduce the death rate from hypertensive disease results from the failure to treat man as a self-directing individual.

Ketogenic action of niacin and the alcohol of niacin. RALPH G. JANES AND I. M. PETERSON. Department of Anatomy, State Univ. of Iowa, Iowa City.

Earlier it was reported (*Soc. Exp. Biol. & Med.* 63: 410, 1946) that a ketonuria was produced in severely diabetic rats after relatively large amounts of niacin were added to their diet (1 g/kg. diet). It has been found in the present study that the alcohol of niacin when given in similar amounts was also ketogenic, but niacinamide was ineffective in producing a ketonuria. In rats with a milder diabetes, it was necessary, in certain cases, to feed high fat diets along with the niacin compounds in order to produce a ketonuria. When the high fat diet was continued but the excess niacin was withheld the urinary excretion of ketone bodies was reduced or disappeared, and reappeared only on the addition of excess niacin. However, two human diabetics were given niacin starting with 150 mg. daily and the dosage was slowly increased up to 1800 mg. without producing any untoward symptoms other

than the usual flushing reaction. The excretion of ketone bodies was not altered in these patients from pretreatment levels.

Relation of blood sugar to spontaneous and insulin induced hunger sensations. HENRY JANOWITZ (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Our recent studies have led us to believe that the gastric 'hunger' contraction and its epigastric pang are a dispensable component of the complex of hunger sensations in man. Because the rôle of blood sugar has been studied in the past only in relation to gastric contractions, the relationship of blood sugar to hunger sensations has been studied in five normal adult females in the post-absorptive state. 1) Spontaneous fluctuations of hunger sensations bear no relationship to minor variations in blood sugar level. 2) The intravenous injection of 0.3 gm. of glucose/kg. of body weight and the resultant hyperglycemia had no effect on spontaneous hunger sensations. 3) The intravenous injection of 0.1 U of insulin/kg. of body weight reproduced the pattern of spontaneous hunger, on the average, 38 minutes later, and 22 minutes after the depth of the hypoglycemia. The insulin induced hunger occurred at a blood sugar level of 45 mg. %, lasted 18 minutes, and subsided at a blood sugar level of 54 mg. %. It is concluded that blood glucose bears no relation to spontaneous hunger sensations, but that hypoglycemia may be operative under abnormal circumstances.

Sensitivity to intocostrin of normal subjects, patients without myasthenia gravis and patients with myasthenia gravis. P. S. JARRETT (by invitation), L. M. EATON (by invitation) AND E. H. LAMBERT. Sections on Neurology and Physiology, Mayo Foundation and Clinic, Rochester, Minn.

The effects of single intravenous injections of intocostrin or d-tubocurarine were studied on 17 normal subjects, 14 patients who did not have myasthenia gravis and 15 ambulatory patients who had myasthenia gravis. In terms of the equivalent dose of d-tubocurarine chloride pentahydrate/kg. body weight, the threshold amounts of drug producing a decrease in performance of the muscles tested varied from normal subject to normal subject in the following ranges: ocular convergence, 8.25 to 33.0; width of palpebral fissure, 33.0 to 99.0; strength of bite, 33.0 to 99.0; strength of grip, 33.0 to 132, and electromyographic response of abductor digiti quinti muscle (Harvey and Masland), 66.0 to 132. The sensitivities of normal subjects remained relatively constant in repeated tests. The gradient of sensitivities of different muscle groups was relatively constant from individual to individual in the

order in which the tests are named. The effects on nonmyasthenic patients were similar to those of normal subjects. All patients who had myasthenia gravis had a greater than normal sensitivity to curare in some muscle group or groups. In some instances, a decrease of performance was noted with doses as low as 1.4 micrograms per kilogram of body weight. Some patients who had myasthenia gravis had muscle groups in which the sensitivity to curare was not greater than that seen in some normal subjects. On 7 patients who had myasthenia gravis the relative sensitivity of different muscle groups did not follow the gradient of sensitivity found in normal subjects. This does not support the concept that a circulating curare-like substance is the sole cause of weakness in myasthenia gravis.

Central neural mechanisms in cardiovascular depressor responses and sympathetic ocular changes resulting from stimulation of the cerebral cortex. A. C. JOHNSON, E. C. HOFF, E. H. GRAY AND D. M. SHOLES. Medical College of Virginia, Richmond.

Observations of autonomic activity resulting from cortical stimulation were made as far back as the days of Hughlings Jackson. In recent years, largely through the impetus of Fulton and others, it has become obvious that autonomic nervous function is closely coordinated with other nervous activity, and is similarly arranged in a hierarchy of functional complexity culminating at the cortical level. We have shown that cardiovascular and ocular responses from the feline cerebral cortex are discrete, localized, and show definite patterns of functional localization. The area from which sympathetic responses are evoked includes the electrically-excitable motor area and most of the frontal lobe cortex. From the cortex posterior to this pressor area as far back as the Sylvian fissure, falls in blood pressure have been elicited. Frequent rises in pressure after these falls suggest a close relationship to the pressor area. Ocular parasympathetic responses could never be produced by cortical stimulation. Vagotomy does not abolish these falls of blood pressure. Transcortical incisions separating pressor and depressor areas or frontal lobectomy likewise fail to abolish these depressor responses, although they are of smaller magnitude. Abolition of the secondary rises by such transcortical incisions implicates association pathways at the cortical level. Post-stimulatory ocular sympathetic responses, frequently evoked from the posterior portions of pressor area, suggest inhibition during the stimulus and subsequent release. The possibility that further developments may permit neurosurgical intervention at the cortical level in hypertensive cases is suggested.

Influence of metabolic inhibitors upon membrane potentials in vivo. ERVIN KAPLAN AND NORMAN R. JOSEPH (introduced by C. I. REED). Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

The membrane potential across the synovial membrane of the dog was determined *in vivo*. Under the influence of metabolic inhibitors, a positive potential was produced at the site of inhibition. Potentials were measured from the interior at the knee joint to the subcutaneous tissue overlying the medial condyle of the femur. (See Joseph, N. R. *et al.*, *Am. J. Physiol.*, 153: 364, 1948.) Standard readings were obtained with 0.15 M. NaCl on both sides of the capsule prior to the intra-articular application of an inhibitor solution. Neutral, isotonic solutions of the inhibitors in NaCl were employed in all instances. Substances which produced positive potentials of varying magnitude included the following: Na_2, S, NaCN, Na azide, Na iodacetate, Na malonate, hydroxylanine, p-chloro-mercurobenzoate, $HgCl_2$·ferric and cupric ions. The cytochrome-cytochrome oxidase system is the principal enzyme complex involved in electron transfer without hydrogen transfer. Therefore, variations in observed potentials may be interpreted in terms of electron transference and potential differences in the cytochrome system. Variations in potential and electron flow may be produced by changes at any point in the transfer systems. These alterations may take place at the substrate, the dehydrogenase systems, the hydrogen-electron transference systems or the cytochrome system. In specific inhibitions the level of the cytochrome oxidase potential may be decreased; in other instances, the cytochrome system may be partially or completely bypassed with hydrogen-electron transfer via alternate pathways.

Effect of massage and preliminary warming-up upon athletic performance. PETER V. KARPOVICH AND CREIGHTON J. HALE (by invitation). Physiology Dept., Springfield College, Springfield, Mass.

It is almost axiomatic with coaches and athletes that a preliminary warming-up is indispensable with a good performance. Two reasons are usually given: the strength and speed of muscular contractions increase and the muscles and tendons 'limber up' and increase resistance for possible injuries which may result from sudden intensive muscular effort. On the other hand, one may meet athletes who either use no special warming-up or gave up its use because they could not see any advantage in this procedure. Laboratory experiments conducted by Asmussen and Bøje showed that preliminary work, diathermy and hot showers increased capacity for riding a bicycle ergometer; massage on the other hand had no effect. Schmidt found that in riding bicycle ergometers, running 100 meters, or swimming 50 meters, all methods used by Asmussen and Bøje

were beneficial. The present investigators were especially interested in the controversy regarding massage. For this purpose, they experimented on 7 college runners using three types of warming-up: 1) preliminary exercise (10 min.); 2) massage (10 min.); 3) light digital stroking (5 min.). The last method was used as a control, involving a possible psychological effect. Immediately after either of these procedures the subjects ran 440 yards. Each type of warming-up was repeated on each man from two to three times. The average time of 20 trials after preliminary exercise was 56.17 seconds; after massage, 55.84 seconds; after digital stroking, 56.50 seconds. Statistical computations showed that the differences between these times were not statistically significant. Additional experiments are in progress.

Changes in the blood following explosive decompression. J. P. KEMPH (by invitation) AND F. A. HITCHCOCK. Laboratory of Aviation Physiology, Ohio State Univ., Columbus.

Determinations of blood gases, hematocrit, blood cell morphology, mean corpuscular hemoglobin concentration and intravascular bubble formation have been made following explosive decompression of dogs. Results show an increased hematocrit which may have been caused by one or more of the emergency measures which increase blood concentration. No significant change was noted in the formed elements of the blood, nor in mean corpuscular hemoglobin concentration. There is therefore no evidence of a water shift between the plasma and the red blood cells or of intracellular bubble formation. No intravascular bubbles were noted 30 seconds after explosive decompression to barometric pressures greater than 55 mm. Hg; bubbles were a frequent finding within 30 seconds at lower barometric pressures and a constant finding after one minute exposure to 30 mm. Hg. Bubbles were noted in superficial arteries within 30 seconds and in veins within 3 minutes after explosive decompression to 30 mm. Hg. Therefore it was thought that intravascular bubbles were formed centrally rather than peripherally; i.e., in the large veins, heart or pulmonary system. The circulation was probably blocked by these bubbles. Determinations of blood gases showed considerable oxygen present after explosive decompression to pressures less than 50 mm. Hg. Therefore, stagnation anoxia and other factors, rather than anoxic anoxia, may be significant in the etiology of the effects of rapid decompression to barometric pressures below the vapor pressure of body fluids.

Quantitative measurement of regional circulation by the clearance of radioactive sodium. SEYMOUR S. KETY (introduced by JULIUS H. COMROE, JR.). Univ. of

(swimming with attached weights), 2) electrical stimulation, 3) anoxia produced by the application of tourniquets, 4) denervation and 5) tenotomy. The results indicated that the fibers of muscles exercised showed an increase in density of mineral ash occurring after definite periods of contraction. The degree of increase in density of mineral ash was found to be proportional to the strength and duration of the activity up to a certain limit. Once a certain degree of increase had occurred, activity for longer periods caused no further changes. The ash of muscle fibers serving as controls was gray in color; the ash of muscle fibers in which an increase occurred was chalky white. Results with electrical stimulation were similar to those obtained with exercise. The fibers of muscles stimulated electrically showed an increase in density of mineral ash occurring after definite periods of contraction, regardless of the method of stimulation. Anoxia produced by prolonged application of tourniquets resulted in little change in electrolytes. Denervation and tenotomy produced a slight initial increase, followed by a decrease in the density of mineral ash in the fibers of the affected muscles as compared with fibers of normal muscle. The color of the ash in the later stages was blue, contrasted with the gray ash of the fibers of normal muscles.

Physiologic limb preference. ELEANOR M. LARSEN (introduced by WALTER J. MEEK). Dept. of Physiology, Univ. of Wisconsin Medical School, Madison.

The extensive cortical areas controlling limb movement would indicate important physiologic significance. The hand motor area is greater than that for the foot. Unilaterality and bilaterality are exhibited from molecule to man. The incidence of human limb preference is unknown. The majority of individuals demonstrate right-handed preference for *certain* responses, but left-handedness has been observed and stated as occurring in from 10 to 25% of the population, suggesting Mendelian ratio. There are 3 reactions for hands and feet: right, left, or bilateral, forming 8 preference patterns. To determine physiologic limb preference an electronic modification of a precedence indicator was employed. The hands, or feet, were placed upon telegraph keys which controlled thyratrons operating electro-magnetic counters. Upon appropriate light stimulus the pair of limbs were lifted as simultaneously as possible. If one extremity reacted within 10 microseconds prior to the other, its precedence was recorded. One hundred effective stimuli were given in 4 complex bouts with rest periods intervening. The hands and feet of 62 healthy young adult women were tested. The physiologic hand reaction generally reflected the usual response, but individuals *changed* in childhood

often revealed the original preference, although they wrote with the educated hand. The results indicate that the majority of individuals are physiologically bilateral in reaction and only a small proportion are significantly right or left in preference. The data suggest that the nerve pathways for limb stimulus-response may be more efficient either bilaterally, or unilaterally, and this neurophysiologic basis determines intrinsic, or physiologic preference.

Analgesia and anesthesia induced by epinephrine. A. LEIMDORFER. Dept. of Psychiatry, Illinois Neuropsychiatric Inst., Univ. of Illinois, and Dept. of Pharmacology, Loyola Univ., School of Medicine, Chicago.

Observations during our earlier experiments about analgesia following intracisternal, (i.c.) injection of epinephrine (epn) and reports of Ivy and co-workers (about analgesia after intracarotid injection of epn) suggested the trial of epn intrathecally for surgical anesthesia. Twenty-six experiments were performed on dogs. Usually pure epn powder (Parke Davis) dissolved in water was used. In some experiments, epn alone was injected ($\frac{1}{2}$ or 1–2 mg/kg) without sedatives. Several minues after the injection, the dogs became quiet and, about $\frac{1}{2}$ hour later, they were asleep (for 1–2 hr.). Cutting the skin and complete laparatomias were performed during which no signs of pain were observed. In other experiments, a small amount of nembutal was injected intraperitoneally (as a kind of basal anesthesia) prior to epn to decrease the amount of epn needed. Several hours after nembutal, the dogs awakened and showed distinct signs of pain already on pinching the skin. At this time, epn was injected ($\frac{1}{2}$ mg/kg.). About 30 minutes later, complete surgical anesthesia occurred. The bloodpressure, ECG and EEG remained normal, the respiration was not depressed, but stimulated. No after effects were seen during the time of observation after the injection (for 2–3 months) although three to four injections were made into the same dogs. However, two dogs died after extremely large doses. No analgesia and no sleep were observed after i.c. ephedrine. Intradermal injection of epn (1 cc. of 1 : 20,000) (guinea pigs) produced local anesthesia.

Absence of increased insulin sensitivity of eviscerated adrenalectomized rats. R. LEVINE, B. SIMKIN AND D. CUNNINGHAM. Dept. of Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

It is a well established fact that intact adrenalectomized animals are very sensitive to the hypoglycemic effect of insulin. The C_{11}-oxy-steroids on the other hand oppose this action of insulin. In order to delimit

the locus of this hormonal interaction, we have compared the insulin sensitivity of adrenalectomized, eviscerated, liverless rats with the sensitivity of normal eviscerated control animals. The data show that the minimum dose of insulin necessary to effect significantly the blood sugar of either group lies between 0.15 and 0.2 u/kg. body weight. That is, in the absence of the liver, adrenalectomy no longer causes an increased sensitivity to insulin. It would seem therefore that the insulin sensitivity of the *intact* adrenalectomized rat is due to the unopposed action of insulin on the viscera, most probably the liver, and not to unhindered peripheral (or muscle) action. These data emphasize again that the primary carbohydrate abnormality of the adrenalectomized animal is a deficient hepatic sugar production.

Metabolism of acetate by the isolated dog gastrocnemius investigated with carboxyl-labeled acetate. NATHAN LIFSON, AKIRA OMACHI (by invitation), H. MEAD CAVERT (by invitation) AND JOHN A. JOHNSON (by invitation). Dept. of Physiology, Univ. of Minnesota, Minneapolis.

The isolated gastrocnemius of an etherized dog was perfused by means of a closed, pump-oxygenator circuit, the animal's own heparinized blood serving as the perfusion fluid. The muscle was stimulated via the sciatic nerve with maximal single shocks at a frequency of 1/sec. for 75–100 minutes. After the anesthetic ether had been removed (or nearly so) by aeration, carboxyl-labeled C^{13}-sodium acetate was administered to give a blood level of approximately 1.8 mM/100 cc. In 3 experiments it was calculated (from:

$$\frac{\text{atom } \% \text{ } C^{13} \text{ excess in respiratory } CO_2}{\text{atom } \% \text{ } C^{13} \text{ excess in administered acetate}} \times 100)$$

that 26–49% of the respiratory carbon dioxide was derived from the administered acetate on the assumption that both carbon atoms of acetate are converted to CO_2 at equal rates. If only the carboxyl carbon were so converted, these values would be reduced by one half. In 2 control experiments in which a rubber tube was substituted for the muscle, relatively trivial amounts of isotope appeared in the 'respiratory' carbon dioxide. Net disappearance of acetate as measured by blood volatile acid determinations was considerable, amounting to approximately 0.9–2.5 mM in the muscle experiments, on the assumption that the acetate of the blood-muscle system is uniformly distributed. In the controls no disappearance of blood acetate was detected. No definitely significant excess C^{13} was found either in the blood lactate at the end of the experiment or in the muscle glycogen. An accumulation of formate in the blood was not demonstrated. From the above data it is concluded 1) that contracting mammalian skeletal muscle can convert at least the carboxyl carbon of acetate to carbon dioxide and 2) that, in all probability, such muscle can effect net disappearance of acetate.

Effects of postganglionic sympathectomy and vagotomy upon the gastrointestinal tract. C. WALTON LILLEHEI. Dept. of Surgery, Univ. Hospital, Univ. of Minnesota, Minneapolis.

Following complete removal of the celiac, superior, and inferior mesenteric ganglia in dogs, there invariably occurs a fulminating diarrhea associated with bloody stools and the passage of increased amounts of mucus. Extirpation of these ganglia, also referred to as prevertebral ganglionectomy, removes essentially the postganglionic sympathetic fibers from the stomach, small intestine and colon and results in a high mortality in the dogs within the first 2 to 3 weeks due to the resulting inanition and to the pathological changes which occur such as severe gastrointestinal hyperemia, hemorrhagic enteritis, colitis, and in $\frac{1}{4}$ to $\frac{1}{2}$ of the dogs the development of typical appearing peptic ulcers located for the most part in the stomach and duodenum, but occasionally as far down as the terminal ileum. These ulcers likewise often progressed to hemorrhage or perforation. The simultaneous excision of the vagi nerves along with prevertebral ganglionectomy prevented in all animals the development of peptic ulcers and gastroenteritis. The essential physiological changes following postganglionic sympathectomy of the gastrointestinal tract are discussed and compared with the effects of preganglionic sympathectomy upon the gastrointestinal tract.

In vivo iodination of tissue protein following injection of elemental iodine. H. J. LIPNER AND S. B. BARKER. Dept. of Physiology, State Univ. of Iowa, Iowa City

Subcutaneous injection of elemental iodine, dissolved in propylene glycol, caused an elevated plasma protein-bound iodine (PI) in normal, thiouracil-treated or thyroidectomized rats. At least some of the PI appears to be thyroxine, since the metabolism of the hypothyroid animals was increased during the period of the injections. The possible rôle of various organs of the animal body in the elaboration of the PI was investigated by comparing the tissue PI levels for kidney, liver, muscle, heart and thyroid with that found at the site of injection. There can be little doubt that iodination of tissue protein took place primarily at the site of the iodine injection, since this PI value was several hundred times greater than any other. The concentration of PI in the kidney was elevated to about the same extent as in plasma, but the liver to a considerably smaller extent. In addition to not being necessary for the iodination reaction being studied here, the thyroid gland appears not to participate, even when present.

Proprioceptive reflexes and muscle coordination. G. N. LOOFBOURROW AND E. GELLHORN. Laboratory of Neurophysiology, Univ. of Minnesota, Minneapolis.

The same functional associations of limb muscles found by Bosma and Gellhorn to respond to stimulation of specific sites in the monkey's motor cortex may be activated on a spinal level by muscle stretch. By means of electromyography it may be demonstrated that the triceps and the flexors carpi show a reflex contraction as a result of passively flexing the elbow. After tenotomizing the triceps, neither it nor the flexors carpi respond to elbow flexion, showing that the response of both depends upon proprioceptive reflexes originating in the stretched triceps. A pull on the tendon of the triceps again elicits activity in both muscles. Stretching the flexors carpi, either by dorsiflexion of the wrist or direct load, excites the same complex. The biceps-extensor complex is activated by elbow extension, even after tenotomizing the extensor carpi. Stretching an extensor carpi by a load on its tendon, or by volar flexion of the wrist also excites the complex. If a reflex be evoked by electrical stimulation of an afferent nerve, it too excites one complex or the other, depending on the nerve chosen. Facilitation of the response of the 'associated synergists' in such a complex results from proprioceptively 'favoring' one or more muscles in it. The 'favoring' has been done by loads applied individual muscles, and by fixation of joints in a position to place certain muscles under stretch. This facilitation has been shown to hold for the semitendinosus-tibialis complex in the hind leg, as well as for the biceps and triceps complexes.

Conversion of lactate to liver glycogen in the intact rat, studied with C^{13}-labeled lactate. VICTOR LORBER, NATHAN LIFSON, HARLAND G. WOOD (by invitation) AND WARWICK SAKAMI (by invitation). Dept. of Biochemistry, Western Reserve Univ., Cleveland, Ohio, and Dept. of Physiology, Univ. of Minnesota, Minneapolis.

The metabolic path linking lactate and liver glycogen has been investigated in the intact rat. $C^{13}H_3C^{13}$-HOHCOONa and CH_3C^{13}HOHCOONa were given with glucose by stomach tube to fasted rats. The resulting liver glycogen was isolated, hydrolyzed to glucose, and the position of the C^{13} in the glucose molecule determined (*J. Biol. Chem.* 159: 475, 1945). The distribution of isotope in the glucose is indicated in the table. Values are in atoms % excess C^{13}.

TYPE OF LACTATE FED	C^{13} IN THE GLUCOSE FRACTIONS		
	carbons 3,4	carbons 2,5	carbons 1,6
CH_3C^{13}HOHCOONa....	0.05	0.27	0.18
$C^{13}H_3C^{13}$HOHCOONa...	0.15	0.60	0.59

The distribution of isotope in the glucose in both types of experiment is consistent with the reactions of glycolysis and the tricarboxylic acid cycle as the main paths for the conversion of lactate to glycogen. Interpreted on the basis of these reactions, the distribution of isotope in the 1,6 and 2,5 carbons of the liver glucose in the α-labeled lactate experiment .18 .27   .27 18. $(C \cdot C \cdot C \cdot C \cdot C \cdot C)$ results from the summing of two   .09   .09 types of isotopic glucose, (a) $C \cdot C \cdot C \cdot C \cdot C \cdot C$, and (b) .18 .18   .18 .18 $C \cdot C \cdot C \cdot C \cdot C \cdot C$, where the superscript actually represents the relative contribution of excess C^{13} made to the final glucose. Glucose (a) is formed directly via the reversal of glycolysis. Glucose (b) is formed from lactate which, via pyruvate and CO_2 fixation, has entered the tricarboxylic acid cycle. Four times as much isotope is seen to have traversed the latter path as the former. Assuming that glycolysis and the tricarboxylic acid cycle are the main pathways of conversion, four mols of administered lactate traverse reactions of the tricarboxylic acid cycle prior to glycolysis for each mol that is converted to glucose directly via glycolysis.

Effect of acute anemia, of anoxemia and of convulsions upon the temperature of the hypothalamus of the cat. HANS LOWENBACH. Depts. of Neuropsychiatry and Physiology, Duke Univ. Hospital and School of Medicine, Durham, N. C.

Thermo-electric measurements were made in the brain of 43 cats under light nembutal anesthesia. Compression of the common carotid artery, either on one side or on both sides simultaneously, produced a prompt and marked increase of the temperature in the hypothalamus and adjacent rhinencephalic structures. The temperature rose usually about ½ of 1°C. within two minutes, but occasionally greater changes were observed. In the thalamus and in other cerebral tissue above the hypothalamus, the temperature increase was smaller and it was delayed in proportion to the distance from the hypothalamus. In and near the cerebral cortex, the temperature fell during occlusion of the carotid artery and rose after readmission of blood. Asphyxia and convulsions induced by various means, produced a comparable increase in heat production in the hypothalamus.

Effect of antihistamine drugs on the flares produced in the skin of normal human beings by burning and freezing. ALEXANDER LOWY AND CHARLES F. CODE. Mayo Clinic, Rochester, Minn.

Lewis and his co-workers have shown that a triple response involving the production of 1) a red spot, 2) a wheal at the site of the red spot and 3) a surrounding

flare is the standard stereotyped response of the skin to minor injury and that the response is due to the liberation of an H-substance which is either histamine or a substance closely resembling histamine. As a means of further elucidating the mechanism of this reaction to injury we have studied the effects of antihistamine drugs on the flare component of the triple response produced by burning or freezing the skin of normal human beings. The flares that we obtained in response to burning were small and the antihistamine drugs had no effect on them. Because of the minimal response that we obtained, definite conclusions regarding the action of these drugs on burning are not being drawn. The results obtained with freezing however were decisive. Large, uniform flares were routinely obtained. These were consistently inhibited by the presence of antihistamine drugs.

Prophylaxis and therapeusis of experimental renal hypertension with purified renin. J. M. MARSHALL (by invitation), HIROAKA MINATOYD (by invitation), R. O. BURNS (by invitation) AND G. E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Previous studies of the effects of crude hog renal extracts on renal hypertension in the dog have shown that the injection of certain renin-containing extracts could prevent the development of renal hypertension following renal artery constriction and could reduce the blood pressure in dogs with an already established hypertension. Antirenin was produced by these extracts, but correlation of antirenin with antihypertensive effect has not been established. In the present study a more highly purified preparation of hog renin was used, both in prophylaxis and in therapy, to determine whether renin was the active agent in crude extracts and to investigate the rôle of renin in the initiation and maintenance of renal hypertension. Hog renin was prepared by a controlled ethanol fractionation technique. Purity of successive lots ranged from 35 to 125 Du (Goldblatt) per mg. nitrogen, averaging about 75. Blood pressures were determined twice weekly by direct femoral puncture. Antirenin titers of serum were determined at monthly intervals. Ten dogs were used in a prophylaxis experiment. After a control period of pressure determinations the dogs were injected daily intramuscularly with purified hog renin. Seven dogs received 10 Du/kg. daily, and 3 received 3 Du/kg. After 3 months the right renal artery was constricted by a Goldblatt clamp. Three weeks later the left renal artery was constricted. Renin injections were continued for 1 month following the second clamping, in surviving dogs. As a control group, 9 normal dogs were bilaterally clamped in exactly the same

fashion. Results in the 2 groups differed markedly. All 9 controls became hypertensive, giving elevations of 30 to 60 mm. Hg. in the first week following the second clamping. These dogs had only mild transient impairment of renal function (maximum blood urea nitrogen values below 60 mg. %). The 10 dogs treated prophylactically showed severe renal insufficiency after the second renal artery constriction, with values of 60 to 250 for b.u.n. Seven of the 10 died in 3 to 9 days in typical renal insufficiency. Three survived and recovered adequate renal function without elevation of blood pressure. Of the 10, only 2 showed any rise in pressure (20 and 40 mm.). These had low antirenin titers (6 DAu/ml.). The maximum antirenin titers of the other 8 dogs ranged from 3–120 DAU/ml.

Production and closure of atrial septal defects in the dog: Observations on atrial pressures. WILLIAM B. MARTIN AND HIRAM E. ESSEX, Div. of Experimental Medicine, Mayo Foundation, Rochester, Minn.

A procedure was devised in dogs which resulted in large atrial septal defects. A knife was introduced into the right atrium through the auricular appendage, the tip advanced to the septum and this structure incised. Right atrial pressures taken by a water manometer averaged 3.8 cm. in a series of 9 normal dogs with open chests. Left atrial pressure in a series of 7 normal dogs with open chests averaged 6.3 cm. H_2O. In 6 animals in which the pressure in both chambers was measured, the left reading exceeded the right by an average of 2.6 cm. H_2O. In a series of 5 animals with proved septal defects in which both right and left pressures were recorded within a few minutes of each other the left exceeded the right by an average of 4.3 cm. H_2O. In 3 of 4 dogs with proved atrial septal defects the right mid-atrial sample of blood exceeded the anterior vena cava sample by 2.5, 5.1 and 5.9 volume % of oxygen. For closing the atrial septal defects a piece of polythene sheeting covered with an inverted segment of vein was introduced into the right atrium and brought into apposition to the atrial defect. Three silk sutures with straight needles were attached to the graft and introduced separately into the right atrium through a small incision. The needles were brought out of the chamber at points in the plane of the atrial septum. The graft became endothelialized within two weeks. The atrial defect closed spontaneously behind the graft within one month.

Effect of prostigmine on intestinal motility in human beings. JOHN M. MCMAHON, CHARLES F. CODE, WILLIAM G. SAUER AND J. ARNOLD BARGEN. Mayo Foundation and Clinic, Rochester, Minn.

A study has been made of the effect of prostigmine on the motility of the bowel in a series of human volunteers.

Detailed observations were carried out on 5 subjects. One of these had undergone ileostomy and 4 had undergone colostomy. The motility of the bowel was recorded by means of a tandem balloon system connected with a glass-spoon manometer which recorded optically on a photographic camera. The balloon system contained both water and air. The recording allowed the determination of the effect of prostigmine on the amount of activity present in the bowel, the types of contractions, their rate, duration and amplitude and the co-ordination between contractions by adjacent segments of the bowel. After the intramuscular injection of 0.5 mg. of prostigmine methyl sulfate an effect on the bowel was noted in from two to eight minutes. This consisted of a definite change of the character of bowel activity. There was a decrease in the number of *type 1* and *type 3* contractions and an increase in the number of *type 2* contractions. The average height of the *type 2* contractions increased and the incidence of co-ordinated *type 2* contractions rose. The proportion of each hour that the bowel was active was no greater after the drug than before. Thus prostigmine shifted motility toward propulsion which was indicated not only in the recordings but also by the expulsion of feces and gas.

Local factors influencing deep rectal temperatures in man. JERE MEAD AND C. LAWRENCE BOMMARITO (introduced by H. S. BELDING). Quartermaster Climatic Research Laboratory, Lawrence, Mass.

In experiments in this Laboratory in which rectal temperatures of men were studied, temperatures recorded at the tip of a 6-inch flexible rectal catheter have been as much as 1°F. lower than temperatures recorded immediately thereafter as shallower insertions. Studies have been conducted in an attempt to illucidate this seemingly paradoxical phenomenon and indicate its influence on rectal temperature as an index of internal body temperature. In 8 individuals studied under a variety of conditions of heat balance and imbalance, temperatures near the tip of a flexible catheter inserted 8 inches through the external anal sphincter were, in almost every instance, lower than temperatures recorded at intermediate points along the catheter. Deviations observed varied from 0.1° to 1.5°F. and were greatest in individuals whose body temperature was falling relatively rapidly (e.g. 2.5°F. in 20 minutes). X-ray films showed the tip of the catheter in a majority of instances to lie near the postero-lateral wall of the pelvis. Adjacent to the terminal portion of the catheter on the pelvic wall lies the hypogastric vein and its branches. These vessels carry blood passing from the surface of the buttocks, the upper legs and external genitalia. When these surfaces were subjected

to temperatures above body temperature the difference in temperature along the catheter was reduced to 0.1°F. When these surfaces were cooled below body temperature the temperature at the tip of the catheter dropped more rapidly then the other rectal temperatures. It was concluded that cooled venous blood was chiefly responsible for the deviations of temperature noted. Temperatures obtained anteriorly in the rectum near the midline should be sufficiently distant from the pelvic walls o as to be uninfluenced by cooled venous blood returning from the surface of the body.

Inhibition of brain dehydrogenases by anticholinesterases M. MICHAELIS (by invitation), N. I. ARANGO (by invitation) AND R. W. GERARD. Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

The pharmacological actions of drugs with a known anticholinesterase activity are commonly attributed to their inhibition of cholinesterase. That effects on function need not parallel this inhibition and that other actions occur have been found by several workers. Yet the report (*Biochem. J.*, 42: 96, 1948) that, of over a dozen purified enzymes tested *in vitro*, only esterases were inhibited by fluorophosphonates has seemed to confirm this limited and specific action. It is often found, however, that inhibitions are lost as systems are purified; even the fluoroacetates, recognized as inhibitors of oxidations rather than of esterase, are inactive on oxidizing enzymes *in vitro* (*J. Biol. Chem.*, 170: 67, 1947). Moreover, other work from this laboratory has demonstrated an inhibition of nerve and brain respiration by anticholinesterases at physiologically effective concentrations. We have, accordingly, examined the action of DFP (di-isopropyl-fluorophosphate), TEP (tetraethylpyrophosphate), and ES (eserine sulphate), all powerful anticholinesterases, and of MFA (methylfluoroacetate), a metabolic inhibitor, on dehydrogenase activity of brain and nerve. The Thunberg methylene blue reduction technique was used and decoloration times obtained for tissue homogenate blanks, tissue plus each of eleven substrates, and tissue-substrate plus each of the inhibitors at concentrations from 10^{-4} to 2×10^{-2} M. Even at the lowest concentration, DFP inhibited the oxidation of succinate, lactate or glucose by about 15%, and TEP inhibited glucose by 25%, while MFA inhibited glucose, citrate or malate 10 to 20%. At 10^{-3} M, DFP inhibited all dehydrogenases, from 90% for glucose, to 5% for citrate; TEP inhibited all but two, succinate and glutamate, glucose being most affected, with 60% inhibition; and MFA inhibition was greatest on malate, 30%, and zero for lactate. ES was inhibitory only at concentrations above 2×10^{-2}, glucose being again most sensitive. The anticholinesterase drugs thus possess

other actions which must be considered in explaining their pharmacology. DFP and TEP are at least as effective in inhibiting brain dehydrogenases as is the non-antiesterase, MFA.

Resistance of explanted gastric mucosa to various chemical and physical agents. JOHN REGIS MILLER, J. F. HERRICK, FRANK C. MANN, JOHN H. GRINDLAY, AND JAMES T. PRIESTLY. Divs. of Experimental Medicine and Surgery, Mayo Foundation and Clinic, Rochester, Minn.

Many methods have been devised to study the gastric mucosa and its secretions since Beaumont reported his classic observations in 1833. In order to study the local effects of various chemical and physical agents on the gastric mucosa, sizeable portions of the fundi of dogs' stomachs were explanted to the abdominal wall. The presence of acid on these vagotomized explants in response to a meal or to histamine stimulation was used as a test of function. Zinc chloride, tannic acid, sodium morrhuate, oil of peppermint, protamine, water soluble vitamin K, quinone and various pituitary preparations were applied directly to the mucosa by means of gauze sponges. The effects of various physical agents including hyperthermia, microwave diathermy, ultrasonics and ultraviolet irradiation on the function of the parietal cells of the explanted mucosa were noted. Gross observations were recorded and when indicated biopsies were taken and microscopic sections examined. Strong escharotics produced widespread destruction of the mucosa, but progressive return of function over a one-year period was observed. The mucosa was found to recover rapidly from the effects of local vasoconstriction, local heating, ultraviolet and bodily temperatures ranging as high as 108°. Ultrasonics caused a temporary suppression of the secretion of the parietal cells.

Clinical and experimental aspects of the use of fluorescein dyes to diagnose central nervous system tumors. GEORGE E. MOORE. Dept. of Surgery, Univ. of Minnesota, Minneapolis.

Fluorescein, a highly fluorescent acid chromagen dye, has been used to help differentiate normal and malignant tissues. The most consistent results have been obtained in the examination of brain tumors. Tumor tissue secured from suspected areas by aspiration needle biopsies are readily recognized by the exaggerated fluorescence observed under ultra-violet light. Both glioma and meningioma groups of brain tumors have been recognized correctly as tumor tissue. The expediency and efficiency of this method is self evident: fewer microscopic sections are required, the time saved is considerable, and the neurosurgeon can check all parts of the needle biopsy for tumor. To date this

procedure has been used in 65 cases; two errors in diagnosis have been made. More recently various iodine substitution products of fluorescein have been synthesized. Attempts to use radioopaque dye (tetra-iodophthalic fluorescein) for the direct localization of brain tumors by roentgenography have not been uniformly encouraging. Radioactive diiodofluorescein (I_{131}) has been employed in an effort to diagnose and localize brain tumors before operation. Dye containing approximately 1 millicurie of activity is injected intravenously and following a 2-hour interval, counts are taken over symmetrical areas of the head by means of a specially shielded Geiger-Mueller counter. The source of greatest activity can then be triangulated. Tumors less than 3 cm. in diameter cannot be detected by our present methods unless they are superficial and surrounded by an appreciable amount of edema. In addition small tumors situated at the base of the brain and near the midline are difficult to localize. The reason for the differential predilection of central nervous system lesions for fluorescein dyes is not at all clear. The so-called blood-brain-barrier plays a part, if not the major role, in the process.

Coagulation time of whole blood as measured in silicone coated tubes before and after various surgical procedures. J. L. MORGAN (by invitation), NELSON W. BARKER (by invitation) AND GRACE M. ROTH. Sections of Internal Medicine and Physiology, Mayo Foundation and Clinic, Rochester, Minn.

An objective method for determining the coagulation time of whole venous blood was designed combining the use of silicone coated tubes with the coagulochronometer as described by Barker and Barker (*Proc. Staff Meet. Mayo Clinic*, 23: 230–233, 1948). The tubes used were screw cap glass vials sealed by bakolite caps and were designed by the Kimble Glass Co. Syringes, needles and tubes were coated with General Electric 'Dri Film 9987'. The mean value for the coagulation time of whole blood as determined by this method employing fifty-one normal subjects was twenty-five minutes. Twenty-five patients were studied by coagulation time determinations done on the day before operation, and the second, fourth, sixth, and eighth days after various surgical procedures. No uniform changes were noted in the coagulation times of these patients on the different days after the surgical procedures. One patient died of a massive pulmonary embolus on the seventh postoperative day and no change was noted in the coagulation time of the blood of this patient. Therefore, by this method, which incorporates a prolonged normal value of the coagulation time of whole blood, no uniform changes of the coagulation time could be detected after surgical procedure and no pre-

dictions of impending venous thrombosis could be made.

Nitrogen balance index and specific dynamic action in rats receiving amino acid mixtures low in isoleucine, methionine or valine. E. S. NASSET AND JOSEPH T. ANDERSON. Dept. of Physiology and Vital Economics, Univ. of Rochester, Rochester, N. Y.

N balance and energy metabolism were determined simultaneously on adult rats fed amino acid mixtures When a 'complete' amino acid mixture, simulating egg protein, was fed after a 7-day 'N-free' diet period, the N balance index was invariably greater than unity. Reducing the DL-isoleucine to $\frac{1}{3}$ resulted in a significant decrease in N balance index and an increase in SDA. A similar reduction in DL-methionine halved the N balance index without affecting the SDA. Neither the reduction of DL-valine to $\frac{1}{3}$ nor the substitution of glycine for glutamic acid in the mixture caused any significant change in N balance index or SDA. The total N requirement for equilibrium on the complete amino acid mixture was computed to be 162 mg. N/day/kg$^{3/4}$. Amino acid requirements were: 1) 6.5 mg. DL-isoleucine N/day/kg$^{3/4}$; 2) 4.9 mg. DL-methionine N/day/kg$^{3/4}$; 3) and less than 5.9 mg. DL-valine N/day/kg$^{3/4}$.

Effect of topically and systemically administered pilocarpine on the denervated iris of the cat. ENID A. NEIDLE (introduced by WALTER S. ROOT). Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.

Twenty-five cats, subjected to unilateral removal of the ciliary ganglion, were used to demonstrate the 'sensitization' phenomenon ('paradoxical pupil constriction') to pilocarpine. The drug was either applied topically into each conjunctival sac at 0.04 mgm. or administered systemically into the femoral vein at 0.4 mg/kg. of body weight. These doses were chosen because the denervated pupil responds by maximal constriction to these doses whereas the normal pupil, which served as a control, showed no response. Certain differences have been observed in the reactions of the denervated iris to topical and systemic administration of pilocarpine and these are briefly: 1) the latent period for the action of pilocarpine given topically is longer than for intravenous administration; 2) maximal sensitization to topical pilocarpine follows immediately after operation while maximal sensitization to intravenous pilocarpine develops six to eight days following denervation; 3) the response of the denervated iris to topical pilocarpine is at all times more intense and long-lasting than is its response to intravenous pilocarpine; 4) the response of the denervated pupil to topical pilocarpine is not significantly altered either with the passage of time or as a result of repeated doses of the drug, whereas, in the same animal, the response to intravenous pilocarpine appears to decrease with repeated dosage. This finding probably cannot be explained on the basis of possible regeneration to the sphincter since in only three of the 25 cats in the series was there any decrease in size of the formerly maximally dilated pupil or any return of light reflex. In addition, the decreased response of the pupil after repeated administration of the drug does not seem to be related to time but rather to the number of previous intravenous doses of the drug.

Regulation of capillary flow in subcutaneous tissue. PAUL A. NICOLL AND RICHARD L. WEBB (by invitation). Depts. of Physiology and Anatomy, Indiana Univ., Bloomington.

The actual characteristics of blood flow through capillaries can only be determined by direct observation of these vessels in a suitable field. In the mammals such aerase are indeed limited. The observations here reported were made on the thin membrane of the bat's wing. Technical details have been reported elsewhere. It is only necessary to emphasize that the preparation allows one to study normal capillary beds in their normal environment. Assuming an adaquate pressure gradient from artery to vein, flow behavior in a given capillary is chiefly determined by three factors: First and most important is the nature of the active vasomotion exhibited by the supplying arteriole and precapillary sphincter. This of course determined the effectual pressure head at the capillary origin. Secondly, the types of interaction between the endothelial tubes and the formed elements of the blood determine largely the magnitude of the capillary resistance. The third factor is the venule behavior which regulates the flow out from the capillaries. Active vasomotion is the result of contraction or relaxation of spirally wound smooth muscle cells along the arterioles and terminal arterioles. There behavior appears regulated by a dynamic equilibrium between the cells and their interstitial environment. No direct nerve connections can be demonstrated, although impulses passing along adjacent fibers do effect their behavior to some extent. The main regulation appears to depend on changes in metabolites in the cells immediate environment. Although each cell is an independent effector the usual condition is a rhythmical contraction and relaxation along an entire terminal arteriole. This produces alternate flow and quiescence in the capillary beyond. It is suggested that this behavior allows alternating periods of outward and inward movement of water and dissolved substances along the entire capillary bed. Thus during the period of constriction the hydrodynamic pressure factor within

the lumin would fall to zero and the only affective force active would be the solvent diffusion pressure (i.e. colloidal osmotic pressure) from interstitial space to capillary.

Protein pattern of liver and intestinal lymph. JAMES T. NIX. Mayo Foundation, Rochester, Minn.

Grindlay and Cain (1947) developed a simple, reliable method of intubating lymphatics with polythene tubing that has made possible a broader investigation of the protein moiety of liver and intestinal lymph. Accordingly, the object of this investigation was to determine average values for the concentration of protein fractions and the rate of protein flow of liver and intestinal lymph in the normal dog under constant experimental conditions. Observations were made on specimens from 13 liver lymphatic cannulations and 10 intestinal lymphatic cannulations. Average values for pure liver and intestinal lymph in the normal dog were controlled by blood studies; by comparison with mixed liver and intestinal lymph from the thoracic duct of the normal dog; by contrast with pure and with mixed liver and intestinal lymph from dogs with liver disease, experimentally produced; by supplemental studies on cisternal lymph from the albino rat. Protein was fractionated by sodium sulfite precipitation; the concentration of protein fractions was measured by Kjeldahl technic.

Time of appearance of flare and wheal after intracutaneous histamine. ERIC OGDEN, P. A. CLOUSE, AND R. V. MURRAY, JR. Dept. of Physiology, Univ. of Texas Medical Branch, Galveston, and Scott and White Clinic, Temple, Texas.

Using the multiple puncture technique of intradermal administration of 1:1000 histamine, the authors undertook to determine what measurements of the histamine reaction would be most reliable and to establish normal values for these measurements in subjects with no evidence of vascular disease. Attempts to measure the response by the size of the flare or wheal were abandoned as unreliable, and final correlation of measurements was limited to the interval between the puncture and the appearance of the response. Seventy subjects were tested, ranging in age from 14 to 76. Five test sites were used: the brow, epigastrium, volar aspect of the forearm, lateral aspect of the ankle and the dorsum of the foot. Results were as follows: the average times for the flare on the brow, epigastrium, and forearm were 21, 25, and 30 seconds, whereas on the ankle and foot the times were 55 and 61 seconds respectively. The average times for the appearance of the wheal were 99, 91, and 96 seconds for the brow, epigastrium, and forearm respectively, and for the ankle and foot 168 and 159 seconds. There was no

significant relationship shown between onset of the flare or wheal and age. There was no correlation between the rapidity of onset of the flare and wheal and skin temperatures. The ankle and foot showed a slower reaction time than the areas on the upper portion of the body—approximately twice as long. This correlates with the findings of other investigators regarding reactive hyperemia in the forearm and foot.

Variations in the oxygen tension and pH of renal cortex. NORMAN S. OLSEN AND HENRY A. SCHROEDER. Depts. of Biological Chemistry and Internal Medicine and the Oscar Johnson Institute, Washington Univ. of Medicine, St. Louis, Mo.

Changes in oxygen tension and pH of renal cortex of nembutalized dogs have been measure *in vivo*. Oxygen tension was followed by the macro-electrode of McCulloch and pH by a modified glass electrode. Both kidneys were exteriorized and an adjustable clamp placed around the left renal artery. A pH and oxygen electrode were placed on the surface of each kidney and changes recorded by means of a D.C. amplifier and inkwriter. On slight constriction of the artery, the left kidney showed a decrease in oxygen tension to about 20% of the control value and a decrease of about 0.2 of a pH unit. This was transient, returning to the control level in about 15 minutes, while the clamp remained tightened. Essentially no changes were noted in the opposite kidney. Complete occlusion of the artery lowered the oxygen tension of that kidney to practically the anoxic state concomitant with a decrease of over 0.5 of a pH unit. In the contralateral kidney there seemed to be a slight significant rise in oxygen tension and no change in pH. These changes remained at their altered levels until the clamp was released, whereupon they returned to their former values. Varying degrees of constriction gave intermediate results. The intravenous injection of 10γ of epinephrine temporarily decreased the oxygen tension of the renal cortex. By constricting the left renal artery the epinephrine effect was lessened in that kidney. On complete occlusion no change in oxygen tension could be elicited by this amount of epinephrine. The response of the opposite unconstricted kidney to the drug was not altered by any degree of clamping.

Effect of intravenous procaine on the heart. M. J. OPPENHEIMER, JOAN H. LONG, MARY R. WESTER, THOMAS M. DURANT. Temple Univ. School of Medicine, Philadelphia, Pa.

Single therapeutic intravenous does of procaine (4 mg./kg.) usually produce no change in the electrocardiogram, heart sounds and blood pressure in anesthetized dogs. An increase in T was found occasionally. At 8–10 mg/kg. T was consistently

changed, usually higher, accompanied by lower voltage in R, increased voltage in S, with formation of 'J' in precordial leads. At 20 mg/kg. QRS was wider, increasing five-fold in width up to 60 mg/kg. Doses of 30 mg/kg. increased PR intervals. At 50–60 mg/kg. ventricular tachycardia resulted. Provided artificial respiration was maintained the above changes were reversible even though blood pressure was very low. At 60–80 mg/kg. ventricular tachycardia, flutter and fibrillation appeared in sequence and artificial respiration was of no avail. One dog with an abnormal heart fibrillated at a much lower dose. Blood pressure, pulse pressure and heart sounds are decreased as QRS widens. This indicates a decreased force of contraction. Spontaneous respiration ceased at 30–40 mg/kg. Serum potassium was unchanged at all doses. The extensive intraventricular block with high doses should be stressed in relation to preexisting arrhythmias and subsequent ventricular fibrillation.

Factors related to voluntary ventilation capacity. A. B. OTIS AND W. C. BEMBOWER (by invitation). Dept. of Physiology, Univ. of Rochester, Rochester, N. Y.

The maximum voluntary breathing frequency for 15 seconds was determined at various tidal volumes in each of 5 subjects. The data are reasonably well described by the equation, $T = bV_T + T_o$, where $T =$ the time for one cycle; $V_T =$ the tidal volume; b and T_o are constants. The minute volume is consequently given by $V_M = V_T/b V_T + T_o$. This indicates that maximum ventilation is performed with the largest possible tidal, but the constants are such that there is little gain with tidals greater than 0.3 the vital capacity. The value of T_o is about 0.08 seconds and may represent the maximum time required for the neuro-muscular mechanism involved to alternate. These equations imply that the mean velocity during a cycle is independent of the tidal volume, but this has not been determined experimentally by a study of breathing patterns. When the above tests were performed using 80% He - 20% O_2 as the respired gas, T_o remained practically unchanged but a was diminished and the maximum ventilation was larger. The resistance of the respiratory passages to gas flow is therefore a factor determining voluntary ventilation capacity. This has been confirmed by simultaneous measurements of alveolar pressure and velocity of flow in 29 subjects for whom the voluntary ventilation capacity and the vital capacity were known. The alveolar pressure necessary to produce a flow of 1 l/sec. was taken as a measure of resistance. $V.V.C.$ showed a negative correlation with resistance ($r = -0.57$) and a positive correlation with vital capacity ($r = +0.79$).

Vascular responses to temperature in the isolated perfused hindlimb of the cat. J. R. PAPPENHEIMER, S. L. EVERSOLE, JR. (by invitation) AND A. SOTO-RIVERA (by invitation). Dept. of Physiology, Harvard Medical School, Boston, Mass.

The temperature of arterial blood supplying the perfused hindlimb of the cat was varied over the range 40 to 5°C. As the arterial blood is cooled from 40 to 25°C. the blood flow at constant pressure is diminished. Below about 25°C. however, the flow increases progressively; at 5–10°C. the blood flow generally exceeds that at 40°C. If the blood vessels are poisoned with NaCN, these vascular responses to temperature are abolished; in the cyanided limb the blood flow diminishes with temperature in proportion to the diminished fluidity of the blood. Evidence will be given that the vessels in the paw and skin respond to cold by vasoconstriction whereas the muscle vessels progressively dilate as the temperature is reduced. At 40°C. the paw accounts for about 30% of the total flow through the limb whereas at 25°C. it accounts for less than 5% of the total flow. Air injected into the femoral artery normally appears in the saphenous vein in the form of large bubbles. However, if the paw is excluded from the circulation or if the blood is cooled, then the intra-arterial injection of air effectively blocks the circulation. Evidently flow in the paw is through large blood vessels, presumably arterio-venous anastamoses, which constrict when exposed to cold blood. The direct constrictor effect of cold on the blood vessels of the superficial tissues (in contrast to the dilator action of cold on the blood vessels of the deep tissues) is of obvious importance to the control of body temperature.

Studies of the anti-heparin compound, protamine. THOMAS W. PARKIN AND HIRAM E. ESSEX. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

It has been known since 1938 that certain protamines will neutralize heparin *in vitro* and in animals. At the time this investigation was started, there were no reports in the American literature concerning the clinical use of protamine for the neutralization of heparin. The purpose of this investigation was to determine by animal studies the toxicity of the protamine, salmine. It is of interest to note that protamines have been proposed recently for the treatment of hemorrhage occurring in certain cases of thrombocytopenic purpura. The intravenous injection of the protamine, salmine, produced toxic effects in the guinea pig, rabbit and dog when large doses were used. The principal toxic effects in the guinea pig were the result of broncho-

constriction and the lethal dose was 6.0 mg/100 gm. of body weight. The principal toxic effect in the rabbit and dog was a fall in the arterial blood pressure. Intravenous injection of salmine also produced constriction of the blood vessels in the ear of the rabbit; this effect was observed through a transparent window placed in the ear. Concomitant with the fall in arterial blood pressure which occurred in the dog after the intravenous injection of salmine, an increase in the portal venous pressure was observed. The fall in the arterial blood pressure which occurred in the rabbit and dog was prevented by the intravenous injection of diphenhydramine (benadryl) shortly before the protamine was injected. This evidence suggests the possibility that release of histamine may be a factor responsible in the production of toxic effects following the intravenous injection of salmine. Intravenous injection of salmine promptly neutralized the anticoagulant effect produced by intravenous injection of heparin in the dog. The anticoagulant effect produced by the intramuscular injection of heparin in Pitkin's menstruum in the dog was temporarily abolished by the intravenous injection of salmine.

Changes in heart rate, blood pressure and electrocardiogram in dogs during diffusion respiration. Thomas M. Parry (by invitation), Joseph N. Spencer (by invitation), Richard W. Whitehead and William B. Draper. Dept. of Physiology and Pharmacology, Univ. of Colorado Medical Center.

Fifteen experiments were conducted in which the changes in the heart rate, blood pressure and electrocardiogram were observed in dogs during diffusion respiration. In addition to a severe respiratory acidosis, the circulation during diffusion respiration is subjected to other abnormal and presumably injurious influences, viz.: an overdose of pentothal sodium, the absence of the respiratory movements and of the 'pulmonary-vascular pump', a high degree of blood concentration and, from the 30th minute onwards, a progressively increasing hypoxemia. Following a 45-minute period of denitrogenation apnea was produced and maintained by the administration of an overdose of 1% pentothal sodium (11 expers.) or by a combination of intocostrin and pentothal sodium (4 expers.). At intervals during the experiment the pH of the abdominal arterial blood was determined and electrocardiogram tracings obtained. The femoral arterial blood pressure was continuously recorded by means of a mercury manometer. The establishment of apnea was not accompanied by significant changes in the heart rate. The systolic blood pressure during diffusion respiration showed an initial fall but thereafter was

well maintained during the period of respiratory arrest. The electrocardiogram revealed the presence of ventricular extrasystoles during the first phase of diffusion respiration which disappeared during the latter phase of apnea. Although diffusion respiration proceeded satisfactorily when a combination of pentothal sodium and intocostrin was used to produce apnea the more severe initial drop in blood pressure and greater difficulty in resuscitation makes its use for this purpose inadvisable.

Analysis of skin, muscle and brachial arterial blood temperatures in the resting normal human forearm. Harry H. Pennes. Psychiatric Institute of New York, New York City.

Temperatures were measured by radiometric and thermocouple techniques in 64 basal, almost nude male and female subjects prone on a hospital bed, the right forearm supported in air. Identical air and wall temperatures ranged from 25.0–27.5°C.; air relative humidities from 45–75%, with linear air veolocity below 20 feet per second. In 10 subjects brachial arterial blood temperatures (average 36.68°) were 0.00 to 0.36° higher than maximum deep forearm temperature (average 36.52°). Skin temperature gradients on the superior surface of the longitudinal axis of the forearm averaged 0.03° per cm. in 14 subjects. Gradients in 17 subjects around the forearm circumference were highly irregular; average maximum difference between any two forearm points was 1.2° for the group. Temperature curves through the complete transverse forearm axis were approximately parabolic. Lack of perfect circular symmetry of the curves precluded individual logarithmic analysis. Theoretical thermal distributions in solid tissue cylinders were plotted by combining the general differential equation of heat flow in homogeneous, isotropic conductors, the Fick principle of physiology, and the Newton Cooling Law. Principal assumptions made were uniformity of rate of tissue heat production, volume flow of blood, and thermal equilibration constant between capillary blood and tissue. Closest approximation of the mean experimental tissue temperature curve was obtained with substituted values of local heat production of 0.0001 and blood flow of 0.0002–0.0005 c.g.s. units, both of which values agree closely with experimental data reported by other workers.

An improved method for recording capacitance changes accompanying the cardiac cycle. Edward Perl (by invitation) and William V. Whitehorn. Dept. of Physiology, Univ. of Illinois, College of Medicine, Chicago.

The use of capacitative changes in a condenser whose field contained the heart to study variations of cardiac volume was first suggested by Atzler and Lehmann (*Arbeitsphysiol.*, 5:636, 1932). Capacity of such a condenser is given by: $C = 0.0885 \dfrac{A\,K}{K(D-T)+T}$ where A = area of plates, K = dielectric constant of the chest, D = distance between plates and T = thickness of the chest. Varying amounts of blood in the heart and vessels in the field during the cardiac cycle change K, thus altering capacity. Difficulties in previous methods of measuring these changes were primarily in adjustment and lack of stability and linearity of response. We have employed newer technics to circumvent these obstacles. The condenser was incorporated in the frequency determining circuit of an oscillator. Its output was passed through a broadband amplifier and a limiter circuit and fed to a discriminator which converted the change in frequency to a D. C. voltage which linearly represented capacitative variations. This was amplified and recorded on a string galvanometer. An oscillator frequency of 10.7 megacycles was chosen to obtain simplicity of circuits, large output and minimum loss current through the poor dielectric of the chest. The apparatus is simple in adjustment and operation and gives consistent, reproducible and linear records in both model and human experiments.

Cerebral projections to cerebellar nuclei with special reference to area 4S. ERIC W. PETERSON, RAY S. SNIDER AND WARREN S. McCULLOCH. Dept. of Psychiatry, Univ. of Illinois School of Medicine, and Dept. of Anatomy, Northwestern Univ. Medical School, Chicago.

By local applications of strychnine to several cortical areas and enough pick-up electrodes on the cortex to define the areas strychninized, the depths of the cerebellum were grouped with bipolar electrodes in a stereotactic instrument and the positions of recordings in it verified histologically. The results indicate that there is a strong projection from areas 4s and perhaps 6 to the homolateral as well as a weaker projection to the contralateral nuclei. If there is a projection from area 4 it resembles that via the motor horn cells to the muscles, for strychnine spikes to the cerebellar nuclei are evoked from 4 only when twitches in the muscles are present.

Intra-arterial changes in pressure before, during, and after exercise. L. H. PETERSON, T. G. SCHNABEL, JR., H. FITZPATRICK AND H. C. BAZETT. Dept. of Physiology, Univ. of Pennsylvania, Philadelphia.

Continuous direct intra-arterial pressures have been recorded during strenuous exercise of four types at four work levels. Recording is done through a flexible catheter in the brachial artery, capacitance manometer and ink writer. Fifteen subjects performed step tests (including one athlete) and in addition 4 subjects performed bicycle tests (including a national bicycle sprint and distance chapion). The Δ value of the product of pulse pressure and pulse rate (rest to exercise) was used as an index of changes in cardiac output in any single subject. Non-athletes show a rise in apparent cardiac output in proportion to the intensity of work. The athlete's circulation increases to a slightly greater degree than the non-athlete's doing step tests and a markedly greater increase when doing the bicycle tests. His diastolic pressure rises appreciably during the step test, but does not rise while riding a bicycle. It appears that the athlete shows an increase in cardiac output which is disproportionately large, with evidence of more effective vasodilatation when doing work for which he is trained. All subjects show a fall in total resistance while exercising. When exercise ceases there is usually a marked, immediate drop in mean pressure with little or no reduction in pulse pressure or pulse rate. This suggests that the simultaneous opening of some additional vascular bed offsets the fall in venous return which would result from the loss of the muscle pump, etc. CO_2 inhalation abolishes this immediate drop. The drop is followed by undulations of the blood pressure during which time the pressure levels and pulse-rate are markedly affected by body movements.

Hyperglycemic activity of pancreatic extracts. I. J. PINCUS, S. A. KOMAROV, W. J. SNAPE AND H. SHAY. Samuel S. Fels Research Institute, Temple Univ. Medical School, Philadelphia, Pa.

Highly purified as well as relatively crude extracts of pancreatic tissue containing insulin are known to elevate blood sugar so as to partially obscure or counteract the insulin activity. Extracts prepared from the pancreas of dogs previously treated with alloxan produce a more marked elevation suggesting that the beta cell does not elaborate this factor. Similar preparations of dog and steer parotid glands fail to provoke hyperglycemia. Our studies on insulin inactivated by the method suggested by Southerland and Cori revealed that intravenous injection of large amounts of inactivated insulin produces a more marked and more sustained rise in blood sugar levels than is provoked by unmodified insulin preparations. Somewhat less of a response is noted after the intraperitoneal injection of either of these preparations with, however, a considerable delay usually present before the peak is reached. Subcutaneous injection minimizes the hyperglycemic activity. Inactivated insulin produces a hyperglycemia in the pancreatectomized as well as the

normal animal; however after hepatectomy and enterectomy only a continued fall in blood sugar was observed. NOVO insulin contains only small amounts of the hyperglycemic factor. Intravenous injection of very large doses of this preparation produces rises in blood sugar of very short duration and inactivation destroys the small amount of hyperglycemic factor present.

Effect of exercise and body position on the venous pressure at the ankle in patients with varicose veins. ALBERT A. POLLACK (by invitation), BOWEN E. TAYLOR (by invitation), EARL H. WOOD AND THOMAS T. MYERS (by invitation). Mayo Foundation, Rochester, Minn.

Direct and continuous measurements of the pressure in the great saphenous vein at the ankle have been made in 13 patients with incompetent veins of the lower extremity when in the supine, sitting and standing positions, and during walking on a treadmill. Pressures were determined by means of a strain-gauge manometer connected to a polythene catheter inserted into the vein through a 17 ga. needle. The average pressure with the patient in the supine position was 8.5 (5.7 to 14) mm. Hg; in the sitting position, 52 (31 to 61) mm. Hg; and in the resting, standing position, 81 (63 to 89) mm. Resting venous pressure at the ankle with the patient in these positions was sufficient to support a column of blood up to the third thoracic interspace at the sternum. This was also found to be true in normal subjects. In 7 patients who had uncomplicated venous insufficiency of the superficial system, walking at 1.7 miles/hr. uniformly produced a decrease in the venous pressure at the ankle, during the first 3 to 6 steps, to an average mean stable value of 44 (34 to 56) mm. Hg which was maintained for the duration of the thirty to sixty second period of walking. In normal subjects similar exercise decreased the venous pressure at the ankle during the first 4 to 12 steps to a mean stable value of 22 (11 to 31) mm. In the patients with incompetent superficial veins, the venous pressure at ankle returned to the resting standing level within 2.8 (1.2 to 5.5) seconds after the walk was completed, as contrasted with the 31 (8 to 57) seconds required in normal subjects. In 6 patients with a history of ileofemoral thrombophlebitis in addition to incompetency of the great saphenous veins the pressure decreased during the first three to five steps to an average mean stable value of 76.5 (47 to 96) mm., and increased to an average value of 3 (1 to 8) mm. higher than the resting standing control value within one second after completion of the walk.

Healing of experimental wounds of the lung. C. B. PORTER, G. M. HIGGINS, AND O. T. CLAGETT, Mayo Foundation, Rochester, Minn.

Experimental studies were made to determine the mode of healing of mammalian lung tissue after surgical injury. The present paper covers studies made on the lungs of the albino rat, the rabbit and the dog. In the rat, simple incisions were made in the anterior lung edge and were allowed to heal without the use of sutures. A second series of incisions in the rat lung were sutured and allowed to heal. Vital staining was used to delineate the phagocytic cells concerned in lung repair in the rat. In rabbits and dogs small wedges of lung tissue were excised, the defects closed with fine sutures, and the healing process studied. In the rat and dog, healing occurred with the formation of a connective tissue scar similar to scars elsewhere in the body. No regenerative phenomena were noted. In the rabbit fibrous healing also occurred but was accompanied by abortive regenerative phenomena on the part of the injured bronchial tree. The end result howerver was a fibrous scar containing no new formed pulmonary tissue.

Increase in irritability of a mammalian sensory nerve following ischemia. E. L. PORTER AND J. L. COLEMAN (by invitation). Dept. of Physiology, Univ. of Texas School of Medicine, Galveston.

Wharton and Porter (*Federation Proc.* 6: 181, 1947, and in press, *Journal of Neurophysiology*) have reported a method by which a segment of mammalian motor nerve (cat peroneal) can be rendered ischemic at will, and repeatedly, and the effects observed, using contractions of tibialis anticus muscle as indicator. The effect of ischemia for the first few minutes was a marked increase in irritability as indicated by higher contractions of the muscle. It has been found possible to repeat this procedure on a sensory nerve (posterior tibial of cat). Reflex contractions of tibialis anticus muscle indicate changes in irritability of the nerve during and after ischemia. These contractions increase in height following the clamping of the blood vessels. Removal of the clamp after 2–3 minutes results in a decrease in reflex contraction height to the original level within the following few minutes. The experiment may then be repeated. If the clamp be left on the vessels, the contraction heights remain high for some 10 minutes and then diminish and disappear during another 10 minutes. An interval of a half hour or more is then necessary before the experiment can be repeated. The results indicate that a sensory nerve behaves toward ischemia just as the motor nerve does. The reflex contraction of tibialis anticus is a part of the flexion reflex. In the acute spinal cat this reflex can only be elicited by stimuli which would be painful to the conscious animals. Our results, therefore, have a bearing on the question of ischemic pain as,

for example, that following the plugging of an artery by a thrombus.

Effect of humidity on the change in body temperature of the albino rat during exposure to low atmospheric pressures. F. H. QUIMBY, N. E. PHILLIPS, B. B. CARY AND R. MORGAN. Dept. of Zoology and Physics, University of Maryland, College Park.

The body temperature of the rat at reduced pressures shows no change when the air supplied to the animal contains oxygen at sea level equivalent. However when employing normal air there is a significant reduction in body temperature with each drop in pressure. A definite relationshp between the partial pressure of oxygen and the body temperature is shown by two factors: first at each reduced pressure the newly established body temperature, once adjusted, is maintained as long as that pressure prevails; and second, when the animal is returned over the same increments to normal pressure the body temperature at each pressure is remarkably close to those established at corresponding pressures maintained during pressure reduction. In moist air the temperature effect of pressure reduction is delayed and less severe. The average fall in body temperature at 260 mm. Hg pressure is 6.1°C. in dry air and 2.9°C. in moist air, the higher temperature in the case of the latter being due to the fact that in humid air the animal can lose little heat by evaporation from the respiratory and external surfaces. This heat saving effect may result in reduced energy metabolism and a decrease demand for oxygen, which in turn may account for the increased resistance which animals in moist air offer to anoxia.

Inhomogeneity of alveolar air. HERMANN RAHN. Dept. of Physiology, Univ. of Rochester, Rochester, N. Y.

Individual alveoli or parts of the lung are neither equally ventilated nor circulated thus producing inhomogeneity of the alveolar gases. It is desirable to predict the relative O_2 and CO_2 concentrations that must exist for the infinite number of ventilation/blood-flow ratios in any part of the lung. By combining the alveolar ventilation equation (Fenn '46) with the Fick equation one finds an expression for the alveolar CO_2 or O_2 in terms of bloodflow and ventilation. Thus for example the

$$\text{alv. p } CO_2 = \frac{.864 \times \text{bloodflow} \times \text{(V-A) } co_2}{\text{alv. ventilation}}.$$

Assuming particular venous blood values it can be shown that each ventilation bloodflow ratio produces a definite alveolar respiratory quotient. Furthermore, all possible simultaneous aiveolar O_2 and CO_2 values must fall upon a definite curve when the O_2 is plotted

against the CO_2. Experimental values agree fairly well with the theoretical ones.

Quantitation of the output of the sweat glands and their response to normal stimulation. WALTER C. RANDALL AND WARREN MCCLURE (by invitation). Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.

Simultaneous observations upon the quantity of sweat produced and the number of functioning sweat glands on a given area provide data from which an estimation may be made of the quantity of sweat discharged by a 'typical' sweat gland in the area. It should be emphasized that the sweat glands are not continuously active but periodically discharge sweat upon the skin surface. During the normal sweating responses of a subject at rest in a warm environment, the average output of the glands of the arms and legs is from .0037 to .0043 mg/min., while that on the dorsal surfaces of the hand and foot is .002 to .003 mg/min. It is not known at present whether this apparent variation represents morphological or functional differences in the responses in these areas, but it is known that similar relationships exist in the larger outputs of the glands when stimulated directly by cholinergic drugs. The preliminary response of the sweating mechanism to the stimulation induced by mild exercise and by heat is to increase the number of functioning glands. If this response proves inadequate to meet the demands of temperature control, further evaporative heat loss may be brought about by an increased output of the individual glands over a given period of time.

Effect of pyrimidines and thiopyrimidines upon liver, nucleic acids and regeneration. DAVID RAPPORT, ATTILIO CANZANELLI, AND RUTH GUILD. Tufts College Medical School, Boston, Mass.

The background of this investigation was the possibility tht administered pyrimidines might tend to increase the nucleic acid content of liver and its regenerative capacity, and that thiopyrimidines might reverse the effects by acting as competitive inhibitors. The relation to the thyroid was also studied. One per cent by weight of the various pyrimidines was added to the food of 3- to 6-month-old rats for periods of 10 or 20 days, following which ⅓ of the liver was removed. After 96 hours the animals were killed. Ribonucleic acid (PNA) and desoxyribonucleic acid (DNA) were determined by the method of Schmidt and Thannhauser, as well as total N and percentage regeneration. Wet wt./dry wt. ratio was also determined; it was unchanged under all the experimental conditions. The chief results were as follows: the administration

of uracil and thymine had no effect whatever. Thiouracil, thiothymine and propyl-thiouracil all reduced the regeneration, and after 10 days raised the total N, which after 20 days of thiopyrmidine returned to normal. Thiothymine reduced (after 20 days) both PNA and DNA; thiouracil and propyl-thiouracil had no effect on these. As we reported previously thyroidectomy resulted in a fall in PNA in the prelobectomized liver, and an equivocal fall in the percentage regeneration; while feeding dried thyroid in large doses (500 mg. daily) increased both as well as the DNA concentration. Neither thyroidectomy nor thiouracil changed this thyroid effect. However, when young animals were thyroidectomized, there was an unmistakable fall in the regeneration, and when dried thyroid was given only in approximately replacement amounts (3.4 mg. daily) the regeneration returned to normal, and PNA to above normal. Moreover, when thiouracil was given together with this amount of dried thyroid the regeneration was again reduced, though not the PNA. We concluded that the thyroid hormone is a regulator of liver regeneration, and that probably its function can be partly taken over in the absence of the gland. We also conclude that thiopyrimidines reduce the regenerative capacity of liver, thereby antagonizing the action of the thyroid hormone, probably by an effect on the enzymatic process in the liver itself. The postulated competitive inhibition of pyrimidines by thiopyrimidines was not proved, but is not ruled out, since nucleic acid pyrimidines may be synthesized in the body from smaller fragments.

Electron microscope studies on tubercle bacilli (BCG) by shadow casting technique. C. I. REED, B. P. REED (by invitation) AND SOL R. ROSENTHAL (by invitation). Depts. of Physiology, and Bacteriology and Public Health, Univ. of Illinois, Chicago Professional Colleges and Cook County Hospital, Chicago.

Chromium shadow casting of tubercle bacilli subjected to various techniques in the preparation of BCG vaccine were examined on the electron microscope. Artefacts were deliberately introduced in order to ascertain details of internal structure. Clear evidence of the existence of a capsule was obtained. Cytoplasm was usually collected in three or four masses suggesting that reproduction by cross-fission might be preceded by clumping of cytoplasm. Polar bodies could be seen in many organisms. The significance of these has not been determined. It is doubtful that branched bacilli exist. Surface contour was such as to indicate that distortion by drying is not a common phenomenon.

Rapid removal of extracellular potassium. ROGER M. REINECKE, CLEON R. HOLLAND (by invitation) AND FRANCIS L. STUTZMAN (by invitation). Dept. of Physiology, Univ. of Minnesota, Minneapolis.

By vivodialysis, using a modified Kolff dialyzer, it was possible to remove a quantity of potassium from the dog approximating or exceeding the total amount in the extracellular fluid within less than five and one-half hours. The animals survived this procedure.

Effect of reduction of barometric pressure on the respiratory rates of acclimatized and unacclimatized rats. O. E. REYNOLDS. Physiology Branch, Medical Sciences Division, Office of Naval Research, Washington, D. C.

Albino rats previously 'acclimatized' to anoxia by exposure to 18,000 ft. in a low-pressure chamber for 1 hr/day for 9 weeks and paired controls (10 rats/group) were exposed simultaneously to decreasing b.p. to a maximum simulated altitude of 36,000 ft. Respirations/min. (r/m) and extent of thoracic excursion were observed visually at 0 and 18, 25, 28, 32 and 36,000 ft. The average r/m of the acclimatized group was more rapid than that of the controls at sea level. The r/m of both groups decreased with increasing altitude, and the thoracic excursion increased concomitantly until the thoracic excursion became maximal (presumably when the tidal volume = vital capacity). After this point (25,000 ft. for controls; 28,500 for acclimatized) the r/m increased. The principal difference between the two groups consisted of a flattening of the r/m curve of the acclimatized group above 30,000 feet while the r/m for the control group was still increasing. If this difference is valid it indicates more rapid attainment of maximal ventilatory efficiency on the part of the acclimatized animals. These results are discussed with reference to human respiratory rate response to altitude.

Pleural reaction to a polythene prosthesis after pneumonectomy in the rat. JOHN RHYDELL, G. HIGGINS AND J. H GRINDLAY. Institute for Experimental Medicine, Mayo Foundation, Rochester, Minn.

Left pneumonectomy was performed in a large series of rats; in some a lung-shaped polythene bag, filled with fluffed cotton and heat sealed, had been placed in the empty pleural space. It was found that there is a benign pleural reaction to such a prosthesis, and that the amount of exudate is not significantly increased over that seen after ordinary pneumonectomy. A neomembrane forms about the polythene bag which gradually increases in thickness, reaching its maximum about one month after operation. In succeeding months, this contracts down to a thin transparent

fibrous rather acellular plaque. Use of the prosthesis effectively prevents shift of the mediastinum with resultant overdistention of the remaining lung. Following ordinary pneumonectomy, the remaining lung rapidly increases in size and weight, so that by one month after operation it weighs almost as much as the normal combined weight of the two lungs. This increase in weight is largely prevented by use of the polythesis bag.

Effects of rutin upon the capillaries with special reference to the bisulfite phenomenon. R. K. RICHARDS AND KENNETH KUETER. Dept. of Pharmacology, Abbott Laboratories, North Chicago, Ill.

Recently considerable interest has been aroused by the effect of certain flavones upon pathologically increased capillary fragility in human beings. Attempts to demonstrate an effect of these substances upon the capillaries in experimental animals have met with considerable difficulties. The present investigations offer a new approach to this problem. It had been shown earlier (*J. Pharmacology* 79: 111, 1943, and *Anesthesia Analgesia*, 22: 283, 1943) that sodium bisulfite possesses a specific ability to increase the resorptive toxicity of epinephrine and procaine from subcutaneous and intramuscular sites if added in concentrations of 0.1 to 0.4%. This increase of toxicity, for which the name 'bisulfite phenomenon' is suggested, is due to an increase of the absorption rate produced by an effect of the sodium bisulfite upon the capillaries at the place of injection. The toxicity of epinephrine HCl by intramuscular injection in rats was markedly enhanced by addition of 0.1% sodium bisulfite. If however the rats were injected with 50 to 75 mg. solubilized rutin intravenously 10 minutes prior to the intramuscular injection of epinephrine the increase of toxicity caused by the sodium bisulfite could be considerably reduced. Such pretreatment with rutin was without effect upon the toxicity of epinephrine hydrochloride solutions as such. The increase of toxicity caused by the addition of sodium bisulfite to procaine hydrochloride solutions was likewise abolished by rutin pretreatment. However, rutin administration also reduced the toxicity of procaine hydrochloride alone. Procaine was shown to exert a dilating effect upon the capillaries which is counteracted by rutin. The conditions necessary for an increase of the toxicity of a drug by addition of sodium bisulfite (bisulfite phenomenon) are discussed and the action of rutin upon capillary permeability is viewed on the basis of these experiments which permit a quantitative approach for the evaluation of rutin-like compounds.

Effect of tetraethylammonium chloride on gastric secretion in the dog. C. R. ROBERTSON AND M. I. GROSSMAN.

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In the present study we have attempted to show the effect of tetraethylammonium chloride (TEAC) on gastric secretion stimulated by sham feeding, mechanical distention, urecholine, histamine and insulin in the dog. In 4 experiments the response to sham feeding was 66.6 mg. HCl/2 hr. When 10 mg/kg. of TEAC was given the response was 3.4 mg. HCl/2 hr. The TEAC presumably blocks the synapse of the pre- and post-ganglionic vagal fibers. In 15 control experiments with insulin the average gastric secretory response in 3 dogs with gastric fistulas was 67.8 mg. HCl/1.5 hr. The response in 13 experiments following the injection of 10 mg/kg. of TEAC was 13.8 mg. HCl. Here, also, the TEAC blocks the vagal ganglia. The gastric secretory response to distention with rubber balloons was decreased by TEAC in 9 out of 11 experiments on dogs with gastric pouches. The average control response in 11 tests was 28.7 mg. HCl/2 hrs. The average response after 10 mg/kg. of TEAC in 11 tests was 12.4 mg. HCl/2 hr. This decrease is due to the TEAC partially preventing the liberation of the gastric hormone in response to mechanical distention. In 6 tests on dogs with vagi intact and 10 tests on vagotomized dogs, histamine induced gastric secretion was not significantly decreased by 10 mg/kg. of TEAC. Urecholine induced gastric secretion was greatly reduced by TEAC in the dogs with intact vagi and reduced to a smaller extent in the vagotomized dogs.

Various proteins and blood protein production. F. S. ROBSCHEIT-ROBBINS. School of Medicine and Dentistry Univ. of Rochester, Rochester, N. Y.

The value for blood protein production of a variety of proteins has been ascertained in dogs rendered anemic and hypoproteinemic simultaneously. Considerable information has been obtained by determining the ratio of plasma protein output to hemoglobin output under these experimental conditions. The majority of proteins tested give values for such ratio below 50%. Egg albumen, lactalbumen, and fibrin among proteins so far tested behave differently. Supplements of some amino acids to egg albumen alter the picture.

Some comparative aspects of the pulmonary arterial pressure. S. RODBARD AND F. BROWN. Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago.

Measurements of the pulmonary arterial pressure in a reptile (turtle), mammal (dog), and bird (chicken) were used as a basis for a study of the comparative physiology of the lesser circuit. Data on the ventral aortic (pulmonary) pressure in the fish were available in the literature. In all these the pulmonary arterial pressure was

low, ranging around 25/10 mm. Hg. However, the systemic arterial pressure varied markedly from class to class. In the fish the systemic pressure was lower than that in the ventral aorta. In the turtle, the systolic pressures were equal in both circuits, but the systemic diastolic was higher than in the pulmonic; therefore the mean systemic pressure was also higher. In the homeotherms the systemic pressure was much higher than in the pulmonary circuit. The pulmonary arterial pressure remains low in the higher vertebrates probably as a result of factors which act to increase the rate of exchange of blood gases with those of the environment. These include: *a*) the enormous pulmonary vascular bed, *b*) the absence of tissue support of the pulmonary capillaries which are exposed over nearly their entire surface to the environment, resulting in *c*) great distensibility of the capillaries which *d*) provide the conditions for little increment in pressure with greatly increased volume. An increased pressure in such a capillary would produce a transudate and result in disturbed function. The complete division of the primitive ventricle into two chambers resulted not only in the separation of arterial from venous blood, but also provided conditions in which the systemic pressure could be raised without directly affecting the pulmonary blood pressure and blood flow.

Histamine as the possible chemical mediator for cutaneous pain. SOL ROY ROSENTHAL AND RALPH R. SONNENSCHEIN. Univ. of Illinois College of Medicine, Chicago.

Previous studies have indicated that histamine, or a histamine-like substance, may be a peripheral mediator of cutaeous pain. By the use of sensitive biological methods, it was demonstrated that irritation of the skin or cornea by mechanical, electrical, or chemical stimuli, below the threshold for injury, is associated with the liberation of histamine or a histamine-like substance; the quantity liberated varies directly with the intensity of the stimulus. In addition, the perfusion of a solution of histamine onto the denuded skin or its intracutaneous injection is associated with painful sensations. As a corollary, it was shown that certain histamine antagonists (phenol ethers), in sufficient subcutaneous dosage, produced a generalized peripheral anesthesia in the dog, monkey, and human; on intracutaneous injection, they produce local anesthesia. It has since been reported that other anti-histamine drugs, of widely varying structure, likewise act as local anesthetics. The present report concerns itself with the determination of the minimum concentration of histamine necessary to produce sensations on intradermal injection. As a control, the actions of acetylcholine, potassium chloride, and adenosine, substances which might conceivably play a rôle in pain mediation were also tested,

alone and in combination with histamine. Twenty-seven adult subjects were tested. All chemicals used for injection were made up in 0.85% sodium chloride with distilled water. For control injections, the same salt solution was used. The subject was put at rest, was not told of the nature of the experiment or allowed to see the injections. A 22-gauge needle on a 2-ml. syringe was gently inserted as superficially as possible into the volar surface of the forearm. No injection was made until the pain from this prick had subsided. About 0.01 ml. was injected, producing a wheal of 2–3 mm. The injections were randomized with saline controls interspersed. There were as many saline placebo injections as of any given solution used. The subject was instructed to report immediately the occurrence of any sensation following the particular injection. This was noted and timed with a stop watch for a minimum of 3 minutes. In some 215 trials, it was found that painful sensations may be produced by the injection of histamine in concentrations as low as 10^{-18} when introduced into the surface layer of the cutis. These findings are taken to indicate the specificity of histamine in the production of cutaneous pain, and to substantiate the postulate that a histamine-like substance acts as a physiological mediator of pain. Acetylcholine and adenosine apparently do not augment the cutaneous pain producing action of histamine. Potassium chloride in dilutions of 1:1000 or over gave no definite sensation. The fact that the production of itching requires a higher concentration of histamine than that necessary for pain indicates that itching is not a 'subthreshold' pain. The least perceptible manifestations of pain are 'prickling,' 'stinging,' or 'tingling.'

External pancreatic secretion in pancreatic fistula dogs. ERIC F. ROUTLEY, JESSE L. BOLLMAN AND JOHN H. GRINDLAY. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

The plan of this investigation is to study pancreatic juice (volume, amylase, lipase, trypsin, specific gravity and total protein) from chronic total pancreatic fistula dogs, before and after vagotomy and sympathectomy, under the following experimental conditons: *a*) fasting; *b*) various diets; *c*) sham feeding; *d*) ingestion of bile, alcohol, olive oil, and HCl; *e*) injection of secretin, mecholyl, histamine, adrenaline, insulin and pilocarpine. This preliminary report, based on some 300 specimens of pancreatic juice obtained from 18 pancreatic fistulae during the past nine months, lacks conclusive data. The 18 successful pancreatic fistulae have all been cannulations of the main pancreatic duct with polyvinyl plastic tubing after avulsion of the accessory ducts; the majority flowing two to four weeks, one lasting eight weeks. Three duodenal explants (an exteriorized button of duodenum containing the main pan-

creatic duct) failed. The difficulty of maintenance of pancreatic fistula dogs seems to have been best handled by returning the pancreatic juice through a polyvinyl gastric fistula to which the pancreatic cannula is connected constantly between experiments. Pancreatic juice is collected on ice and enzyme activity is preserved by adding an equal volume of glycerin. Volumes are greatest after meals, secretin, histamine and alcohol. Enzymes (amylase, lipase and trypsin) are highest after pilocarpine, olive oil, sham feeding and insulin hypoglycemia. The three enzymes tend to run parallel, however, for example, a fat meal may elevate lipase more than amylase and trypsin. Specific gravity and total protein determinations have been unrevealing.

Sweat gland activity of dogs before and after cinchophen-induced ulcers. A. H. RYAN AND L. M. WIDROW (by invitation). Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago, Ill.

The sweat glands of the dog have had very little study. Electrical resistance of the skin is a sensitive method of measuring changes in sweat-gland activity. It seemed that a study of the effect of cinchophen on foot pad resistance might shed light on its action on the sympathetic nervous system or on this type of gland. Resistance was measured daily by placing the feet in a circuit with 2 dry cells, a variable resistor and a microammeter. Cinchophen was given orally, 2 grams daily, 6 days per week, and measurements were generally made 18 to 22 hours after the preceding dose. Ulcers were produced in 9 normal dogs, and 4 with lumbar preganglionic sympathectomy. The hind-foot pad resistance was significantly decreased by cinchophen in each group, a response indicating increased sweat gland activity. Resistances lower than on any control day occurred within the first 4 days in 85% of the dogs. The following table shows the effects of both sympathectomy and cinchophen on pad resistance.

DOG	BEFORE SYMPATHECTOMY	AFTER SYMPATHECTOMY			DAYS OF SURVIVAL
		Mean—week before cinch.	Mean—week after cinch.	Change in percentage	
Ba.......	5575	75750	5280	−93.0	45
Fl.......	2800	65630	14875	−77.3	21
An.......	150000	1256660	38975	−96.9	27
Sp.......	10800	532500	64600	−87.9	8
Mean....	42293	482635	30933	−88.8	

The fore legs showed, in general, similar changes, but the results were not so clear cut. Cinchophen, therefore, has a demonstrable action on the sweat glands

which, on the basis of the present evidence, may be direct or indirect or both.

Effect of para-aminobenzoic acid on the metabolism and excretion of salicylate. ROBERT M. SALASSA AND JESSE L. BOLLMAN. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

Following a report in 1946 that the oral administration of para-aminobenzoic acid increased the plasma salicylate levels in man, an attempt was made to determine the mechanism by which para-aminobenzoic acid produced this effect. The salicyl fractions excreted in the urine and the plasma salicylate levels were determined in man and in the dog following the ingestion of sodium salicylate with and without para-aminobenzoic acid. In the dog the administration of para-aminobenzoic acid did not elevate the plasma salicylate value and did not alter the excretion of salicylate in the urine. In man the administration of para-aminobenzoic acid appeared to interfere with the conjugation of glycine with salicylic acid and resulted in a marked decrease in the quantity of salicyluric acid appearing in the urine. Kapp and Coburn have shown that man normally excretes about 80% of ingested salicylate in a form that contains an intact salicyl radicle and that approximately 50% of this is salicyluric acid, 25% salicyl glucuronate and 25% free salicylate. Para-aminobenzoic acid administration did not alter the excretion of the free salicylate or salicyl glucuronate fractions unless the *p*H of the urine was changed. When the urine was made strongly alkaline by giving large amounts of sodium bicarbonate with the para-aminobenzoic acid, the excretion of the free salicylate fraction increased enough to completely mask the effect of the decrease in excreted salicyluric acid on the renal clearance of total salicylate. The administration of para-aminobenzoic acid also appeared to interfere with the conjugation of benzoic acid with glycine as indicated by a decrease in the quantity of hippuric acid excreted in the urine in four hours following the ingestion of 6 gm. of sodium benzoate. These effects of para-aminobenzoic acid are temporary and reversible.

Mechanism of apnea induced by electrophrenic respiration. STANLEY J. SARNOFF. Harvard School of Public Health, Boston, Mass.

A new type of artificial respiration has been developed which depends upon the surgical insertion of a single electrode around one or both phrenic nerves and the application of an electrical current to the nerve. By means of a rotating potentiometer the voltage is made to vary between 0 and about 3 volts in such a way as to cause the diaphragm to contract and relax in a smooth manner. The revolution rate of the rotating potenti-

ometer determines the rate of respiration and is readily adjusted. Since the tidal volume is (within satisfactorily wide limits) in direct relation to the peak voltage applied, the depth of respiration and minute volume are likewise easily controlled. The technique is capable of maintaining normal blood gas tensions in the absence of spontaneous respiration in the cat, dog, rabbit and monkey. An interesting by-product of the development of the technique was the observation that spontaneous respiration ceased immediately after the onset of electrophrenic respiration. This suppression of spontaneous respiration is reflex in nature since after vagotomy it no longer occurs.

Thermal conductance of the colonic wall; magnitude of changes during peristalsis, graded hemorrhage and re-infusion. H. SCARBOROUGH, M. ELKIN, H. A. BLISS, H. W. PARK (by invitation), AND E. M. LANDIS. Dept. of Physiology, Harvard Medical School, Boston, Mass.

Thermal conductance in the colon of anesthetized dogs was measured during hemorrhage re-infusion and death to determine whether heat transfer was sufficiently rapid and consistent to serve as a continuous measurement of intestinal (splanchnic) blood flow in unoperated animals and in man. The thermal flowmeter consists of a hollow water-tight lucite chassis, 10 x 1.7 cm., carrying a wire coil which provides a constant known output of heat distributed throughout 34 ml. of water with which the thin rubber balloon enclosing the whole instrument is inflated. The water is mixed by a pulsating pneumatic device. Mounted near the middle of the chassis is one end of a 6–10 unit thermopile which with the heater, is protected from contact with the balloon by a metal guard. The other junctions of the thermopile are mounted on the surface of a solid lucite tip 5 x 1.2 cm., so as to be in contact with the gut wall, and hence at local body temperature when the tip is inserted 25 to 30 cm. up the colon. From the output of heat (e.g. 0.288 cal/sec.) between the temperature of the tip and the water in the balloon (e.g. 1.644°C.) as recorded continuously by an ink-writing potentiometer, the overall thermal conductance, C = *cals/sec.* In rapidly flowing water the C for such instruments was 0.600 to 0.700 cal/sec/°C.Δt. The overall C was 0.150 to 0.250 in the colon of living dogs and less than 0.100 in the colon of dead dogs. When heat was removed from the balloon mainly by conduction (wrapped in cotton-wool in water bath) C lay between 0.070 and 0.090, and by convection (bare in still water) 0.240 to 0.270. Peristalsis, always recorded throughout, usually increased C, whether due to movement artefact or active hyperaemia cannot at present be said. Atropinization

produced relatively constant C for long periods. Graded hemorrhage in normal or atropinized dogs decreased C often in stepwise fashion; re-infusion increased C rapidly and conspicuously.

Patterns of cutaneous hyperalgesic points obtained by stroking the trunk with a sharp stylus. ALFRED A. SCHILLER. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Light linear centripetal stroking with a sharp stylus of the anterior and posterior surfaces of the trunk in an approximately horizontal plane may elicit a *point* of hypersensitivity along the path of the stroke. When the trunk is systematically stroked, the hypersensitive points tend to bilaterally parallel the midline, lying 2–4 cm. laterally. Over the back another array of points angle bilaterally from the upper thoracic spinous processes to the roots of the scapulae, and then run obliquely caudad to join the paramidline points in the region of the lumbar vertebrae. The only constant and appreciable deviation from the bilaterally symmetrical representation of hypersensitive points occurs in the region of the precordium where they are elicited farther to the left, frequently outlining the left border of the underlying heart as determined roentgenographically. With the exception of the precordial deviation the pattern of sensitive points obtained by stroking closely parallels the anatomical distribution of the sites of superficial emergence of the cutaneous branches of the somatic spinal nerves. The painful quality of the sensation and the latency in perception implicates the pain receptors as the principal mediators of the stroking hyperalgesia phenomenon. It is postulated that point hyperalgesia results when adjacent cutaneous receptors are rapidly and consecutively stimulated (stroking), permitting delicate sensory discrimination of densely populated receptor areas from sparsely innervated regions. In the precordium visceral (cardiac) afferent activity may raise the CES and lower the stimulus threshold in the same cutaneous segmental districts to account for the lateral deviation described.

Analysis of the nerve membrane current-voltage characteristic as a non-linear 'impedance'. OTTO H. SCHMITT. Dept. of Physics, University of Minnesota, Minneapolis.

It has been shown that much detailed information concerning the electrical properties of the excitable nerve membrane can be deduced by careful study of the exact shape of the action potential and its first two derivatives. Values for membrane current, charge, resting potential, and electric energy release during excitation are among the quantities which may be evaluated. By identifying the membrane static

capacitive charging current with the first derivative potential form it is possible to isolate the active non-linear impedance portion of the membrane excitation cycle and thus approach closer to a direct measurement of the energy yielding process in the membrane.

Relation between local polarization and spike height in single medullated nerve fibers. GORDON M. SCHOEPFLE AND JOSEPH ERLANGER. Washington Univ. School of Medicine, St. Louis, Mo.

A quantitative investigation of the relation between local polarization and spike height in single medullated nerve fibers was confined principally to the most irritable fiber in the phalangeal nerve of the green frog. Electrode alignment is indicated as follows: anode of the stimulating circuit, stimulating cathode, grounded lead and polarizing electrode in common, distal recording electrode and finally, the distal polarizing electrode, maintained at least 10 megohms above ground through the polarizing circuit. Anodal increments amounting to 80% of normal spike height and cathodal decrements of 60% were obtained with currents just short of blocking intensity. The relation between spike height h and polarizing voltage v conforms to the equation $h = h_0 \pm kv$ in which h_0 is the normal spike height and k is a constant depending only on direction of membrane current flow. The membrane resistance thus appears to be linear, but rectification is manifest in 24 of the 27 fibers investigated, the k factor being the greater for inwardly directed currents. The tangent ratio, or ratio between the two k values for each fiber was found to be unchanged on subjecting the nerve to 95% oxygen and 5% carbon dioxide or to Ringer's solution containing twice the usual amount of potassium, or to crushing under the distal polarizing electrode. Differences in extent of rectification are apparently not correlated with seasonal variations.

Rôle of facilitatory reticulo- and vestibulo-spinal systems in maintaining spasticity. L. H. SCHREINER (by invitation), D. B. LINDSLEY AND H. W. MAGOUN. Depts. of Anatomy and Psychology, Northwestern Univ., Medical School, Chicago, Ill.

The basic feature of the spastic state is an exaggeration of spinal stretch reflexes which are most pronounced and persistent in the antigravity muscles. Requisite to the appearance of spasticity is the elimination of central suppressor influences, release from which permits stretch reflexes to become exaggerated. Equivalently requisite is the maintained and now unopposed influence of central facilitatory systems, including the reticulo and vestibulo spinal systems, for their influence is the actual factor responsible for reflex exaggeration in spasticity. From this point of view, the therapeutic relief of spasticity in clinical conditions, might find a logical point of attack in reducing the activity of the central facilitatory systems which are responsible for maintenance of the spastic state.

Pyruvate metabolism in colpidium campylum. GERALD R. SEAMAN. Biological Laboratory, Fordham Univ., and Marine Biological Laboratory, Woods Hole, Mass.

It has been shown that *Colpidium* is capable of synthesizing lipids from protein (*Biol. Bull.* 94: 29). Since pyruvate, through the tricarboxylic acid cycle, is the link between protein and carbohydrate metabolism, a study of this cycle was made in *Colpidium* as the first step toward the elucidation of the pathway of lipid synthesis in this organism. Pyruvate is rapidly metabolized; 12.5 millimoles are utilized in 4 hours by 25×10^4 cells. Malonic acid inhibits the pyruvate effect (increased oxygen consumption and pyruvate utilization). The malonate effect is overpowered by the addition of fumarate. Succinate is formed upon incubating cells with fumarate, pyruvate, and malonate. The amount of α-ketoglutarate formed from pyruvate is increased four-fold upon the addition of fumarate. When 10 millimoles of oxaloacetate are incubated with 46×10^4 cells, in four hours there is recovered 0.3 millimoles of fumarate, 0.8 millimoles of malate, 3.6 millimoles of pyruvate and 1.7 millimoles of lactate. All the oxaloacetate added is utilized, none being recovered. The transamination system, pyruvate + glutamate = alanine + α-ketoglutarate occurs in *Colpidium*. It has not been possible to recover aceto-acetate when cells are incubated with pyruvate and ammonium chloride.

Gastric and esophageal secretion in the frog. MARY SHELDON (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Heidenhain and other early investigators believed that in the frog the gastric body cells produce acid only, all pepsin coming from the esophageal glands. More recent investigators including Bensley and Friedman claim that both acid and pepsin are produced by the same gastric body cell. The object of this study was to confirm one of these views. In the stomach two kinds of mucous cells were found but no canalicular apparatus could be demonstrated in the body cells to show that they were typical parietal cells. Large heavily stained zymogen granules were found completely filling the esophageal gland cells. Small lightly stained granules were scattered in the gastric gland body cells in the fundic portion of the stomach. Microscopic examination of fresh gastric mucosa from a frog injected with neutral red showed acid secreting cells excreting the dye in some areas. Pepsin deter-

minations made from mucosal extracts and secretions showed high peptic activity in the esophagus and some in the stomach. Histamine increased the pepsin concentration of juice from the esophagus only, but caused some increase in volume of gastric secretion and a large rise in total acidity. No decrease in pepsin was found in the gastric juice of frogs after continuous histamine induced secretion for three days which indicates that the stomach does secrete pepsin. Thus these experiments support the view that the one type of cell in the body of the frog gastric glands secretes both acid and pepsin.

Experimental miliaria in man: II. Production of sweat retention anidrosis and vesicles by various kinds of injury. WALTER B. SHELLEY, PETER N. HORVATH (by invitation), FRED D. WEIDMAN (by invitation), DONALD M. PILLSBURY (by invitation).

A study has been made of the effects of various agents on the human sweat-gland apparatus when applied locally to the skin. They are as follows: 1. iontophoresis; 2. ultraviolet light; 3. heat; 4. solid CO_2; 5. maceration; 6. adhesive tape; 7. $AlCl_3$ 20%; 8. soap; 9. fat solvents. No immediate alteration was noted in the normal sweating processes when the subject was stimulated to vigorous sweating by means of heat. However, within three to five days definite changes were observed within the areas treated. They varied from slight to marked anidrosis, and in the areas of greatest anidrosis, small clear, superficial vesicles developed. The vesicles did not appear in areas in which the secretion of sweat was locally inhibited by atropine. These phenomena could be demonstrated repeatedly over a period of one to two weeks, after which the sweat-function returned to normal. Microscopic study revealed tiny plaques of para and hyperkeratotic stratum corneum in the treated areas. The vesicles were situated entirely within the stratum corneum and serial sections demonstrated that they were directly connected with the sweat ducts. It would appear that the factor common to all stimuli applied is that of minor irritation, and the plugging of the sweat-gland apparatus is part of the response of the skin to this irritation. Evidently various kinds of stimuli can provoke the phenomenon of sweat-retention anidrosis.

A new method to measure elastic properties of skeletal muscle in situ. ERNST SIMONSON, JOSEPH BROZEK AND ANCEL KEYS. Laboratory of Physiological Hygiene, Univ. of Minnesota, Minneapolis.

The method is based on the measurement of the deformation of a muscle *in situ*, produced by a falling hammer striking the surface of the muscle. Evidence is presented that the measurement of the total contact time between hammer and muscle, and of the rebound give a fairly good approximation of the actual deformation curve. The contact time between hammer and a metal plate in contact with the muscle is measured electrically. During the contact, a condenser is charged from a battery and later discharged into a ballistic galvanometer so calibrated that the contact time can be read from the scale in σ. The rebound is measured mechanically on a scale as a quotient of fall height : rebound height. The effect of hammer weight, fall height, size and shape of the contact plate was studied and the optimum conditions for the standardization of the method are demonstrated in two models, one for measurement of biceps muscle elasticity in sitting position, and the other one for measurement of leg muscle elasticity in lying position. Provisions are made for variation of muscle length and tension. The method is simple, the readings can be made within a few seconds. The size of the 'chance' variations is small compared to the changes produced by alterations in muscle tension. The method is sensitive enough to record small variations of muscle tension, and the time of contact is too short for interference of voluntary or reflex alterations of muscle tension.

Observations on the curare-like action of thiamine. JAY A. SMITH (by invitation), PIERO P. FOA AND HARRIET R. WEINSTEIN (by invitation). Chicago Medical School, Chicago, Ill.

When thiamine is injected intravenously in large doses (50 mg/kg. or more) bradycardia, hypotension, vasodilation, and inhibition of respiration are produced. These effects are transitory if artificial respiration is provided. Recent experiments have shown that thiamine exerts neuromuscular block typical of curare, and that, conversely, the symptoms of thiamine toxicity can be produced by curare preparations, namely into costrin (Squibb) and D-tubocurarine (Squibb). Thiamine, 150 mg/kg., is approximately equivalent to D-tubocurarine, 0.15 mg/kg. Except for the great difference in dose, the results are similar; hypotension may last longer with thiamine, whereas the neuromuscular block may last longer with D-tubocurarine. In experiments with frogs, it was found that thiamine exerted typical curare effects. Intocostrin was found to be about 200 times as potent as thiamine on weight basis. In experiments on unanesthetized dogs, both thiamine and D-tubocurarine produce identical symptoms, namely, ataxia, inability to stand, staggering movements and exaggerated reflexes; D-tubocurarine is about 1000 times as potent as thiamine under these conditions. In other experiments the thiazole moiety (4-methyl-5-betahydroxyethyl thiazole, Merck and Company) was found to have curare-like action similar

to thiamine. It was only about one fifth as potent as thiamine in this respect. We conclude that when thiamine is given by rapid intravenous injection in large doses, its toxic effect is predominantly due to its curare-like action.

Intravenous glucose tolerance tests in aged males. LUTHER E. SMITH AND NATHAN W. SHOCK. Institute of Experimental Biology and Medicine, National Institutes of Health, Bethesda, Md., and Baltimore City Hospitals, Baltimore, Md.

Intravenous glucose tolerance tests were done on 68 males in various age groups. There were 14 in the 20–29 year group, 12 in the 50–59 year group, 13 in the 60–69 year group, 17 in the 70–79 year group, and 12 in the 80–89 year group. All subjects were selected with history and physical examination. No subject was used who had a history of diabetes, jaundice, or glycosuria or who manifested edema, jaundice, any type of liver disease or glycosuria. A fasting femoral arterial blood sample was drawn, and 50 cc. of a 50% glucose solution in distilled water was injected into the right or left basilic vein. Arterial blood samples were drawn at 5 and 10 minutes after the start of the injection and every 10 minutes thereafter for 2 hours with a final specimen drawn at 2½ hours. The blood was heparinized and stored in ice, and at the end of the experiment true blood sugar was determined by Nelson's modification of the Somogyi precedure. The mean fasting value varied between 79–82 mg.% for all the groups with no significant difference between any group. There was a progressive increase in the mean time in minutes to return to the fasting level for each group with a significant difference at the 1% level between the 20- to 29-year group and the 70- to 79-year group, and between the youngest group and the 80- to 89-year age group.

On the maintenance of spontaneous activity within the cerebellum. RAY S. SNIDER AND EARL ELDRED (by invitation). Northwestern Univ. School of Medicine, Chicago, Ill.

Preliminary studies on the mechanisms involved in the maintenance of the fast spontaneous electrical activity of the cerebellum have been made upon cats, either decerebrated or under various depths of barbiturate anesthesia. The electrical activity was amplified thru Grass Model III amplifiers and was observed directly on a three-channel cathode ray oscilloscope. That the activity is not dependent upon driving mechanisms outside the cerebellum is shown by the fact that it continues after bilateral destruction of all cerebellar peduncles. That it is not dependent upon a cerebellar-cortex to cerebellar nucleus back to cortex circuit is shown by the fact that it continues following destruction of all cerebellar nuclei. It is concluded that all mechanisms necessary for the maintenance of this activity are located within the cerebellar cortex, and evidence is presented to show that the responsible units are either the granule cell plus glomerulus and/or the Purkinje cell.

Blood and tissue lipids in the chick fed cholesterol in various forms. J. STAMLER, C. BOLENE, E. LEVINSON, M. DUDLEY AND L. N. KATZ. Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

Five groups of 5 Leghorn cockerels each, 5–8 weeks of age, were fed *ad libitum* for 10 weeks on a commercial chick starter mash supplemented with 2% cholesterol in various forms, including: *a*) amorphous cholesterol; *b*) crystalline cholesterol; *c*) cholesterol dissolved in ether and re-evaporated after admixture with the mash; *d*) crystalline cholesterol with cottonseed oil. In addition a control series of 5 birds with no supplement was run. Food intake and bird weights were recorded periodically. Plasma cholesterol (total and free), phospholipid and total fatty acids were measured at regular intervals. Liver, aorta, heart, kidney, intestine and carcass were analyzed at the end of 10 weeks for total and free cholesterol. 1) *Groups a, b* and *c* (cholesterol without oil) responded similarly, developing a progressive moderate hypercholesterolemia and hyperlipemia. *Group d* (cholesterol with oil) developed a far more marked and rapidly progressing hypercholesterolemia and hyperlipemia. In all groups the plasma free cholesterol ratio remained unchanged. The percentage increase in plasma cholesterol in all cholesterol-fed groups exceeded significantly the percentage rise in phospholipid and neutral fat. 2) Early atherosclerosis of the aorta and other great vessels was noted grossly in all cholesterol-fed groups, its extent and severity being significantly greater in *group d* (cholesterol with oil). 3) Tissue cholesterol analyses reveals an organ lipoidosis in all cholesterol-fed groups, which can be generally related to the blood cholesterol levels. The increase in lipid in the various tissues was not uniform, being much greater in the aorta and liver. Accumulation of esterfied cholesterol was the characteristic feature of the organ lipidoisis. The occurrence of atheroma would appear to be related to: 1) the hypercholesterolemia; 2) the apparent disturbance in the ratio of plasma lipid fractions; 3) the organ lipoidosis, particularly of the liver and aorta; 4) the apparent disturbance of the normal tissue cholesterol esterification ratio.

Pressure and activity recordings along the gastrointestinal tract in man. F. R. STEGGERDA AND W. C. CLARK (by invitation). University of Illinois, Urbana.

It was previously reported that satisfactory pressure and activity recordings of the lower colon in man could be made with a water manometer connected to an open-tipped tube inserted five inches beyond the anal sphincter. The method was satisfactory except that plugging of the tube occurred when the colon was not completely empty, or the fecal material thin and watery. With this objection it is obvious that the technique could not be used in recording pressures in other places along the gastrointestinal tract because of the fluidity of its contents. Recently a water capsule (1 cm. long and .5 cm. in diameter) attached to the end of a rubber catheter connected to an electronic recording pick-up was devised which records pressure and activity along the gastrointestinal tract continuously without any interruptions. A comparison between the open-tipped water mamometer and the closed water capsule electronic recording method showed that pressures in the colon of man with the latter method is definitely higher than those recorded by the water manometer. Recording of stomach pressure and activity as well as the changes that occur while the water capsule is passing through the pyloric sphincter region and entering the small intestine were made. Changes in position while lying horizontally on a cot do not significantly alter pressures in the gastrointestinal tract.

Effect of increased carbon dioxide in inspired air on gastric emptying in dogs. J. CLIFFORD STICKNEY, DAVID W. NORTHUP AND EDWARD J. VAN LIERE. Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown.

The normal gastric emptying time of 4 dogs was determined fluoroscopically after intubation by stomach tube of 50 ml. of a suspension of $BaSO_4$ in 20% gum acacia solution. Immediately after intubation, the dogs were kept in a well ventilated chamber in which they were at other times exposed to increased concentrations of CO_2 in the inspired air. The effect of CO_2 was determined by placing the dogs after intubation into a chamber through which were led mixtures of CO_2 in O_2 of the desired concentrations. Twenty-five determinations of gastric emptying were made while the dogs were exposed to concentrations of CO_2 in the inspired air ranging from 6.6–12.3%. Twenty control observations were made on the same dogs. The responses to CO_2 were rather variable, not only in the individual dog but in the group as well. When the CO_2 range was 6–8%, the average increase in gastric emptying time varied from 15 to over 100% in the 4 dogs; between 8 and 10% CO_2 the increase was 67 to over 288%; between 10 and 12% CO_2 the increase was 73 to over 300%. The delay in gastric emptying

appeared to be proportional to the concentration of CO_2 in the inspired air over the range studied.

Influence of estrogens on x-ray toxicity. R. L. STRAUBE (by invitation), H. M. PATT AND M. N. SWIFT (by invitation). Argonne National Laboratory, Chicago, Ill.

It has been reported that a single intramuscular injection of alpha estradiol-benzoate nine days prior to irradiation decreased radiotoxicity in male Swiss mice, whereas estrogen administered on the day of irradiation potentiated sensitivity (*Endocrinology* 32: 161, 1943). We have extended these findings and initiated investigations designed to elucidate the mechanism of this estrogen effect. We have observed a similar protective estrogenic effect in both male and female mice of the CF₁ strain with either alpha estradiol-benzoate or the synthetic estrogen Benzestrol (Schieffelin), 0.1 mg. alpha estradiol-benzoate or 1.0 mg. of Benzestrol, 10 days prior to x-irradiation (500 *r*) giving essentially complete protection. When the estrogen was given five days before irradiation a probably significant but somewhat lesser effect was evidenced. Administration at the time of irradiation gave equivocal results. Since the estrogen effect may be non-specific, other steroids are being investigated. Preliminary experiments indicate that progesterone (0.1–0.2 mg/25-gm. mouse) has little if any protective action. Pilot studies indicate that a single dose of estrogen alone (0.1 mg. I.M.) has no appreciable effect on the weight of spleen, inguinal nodes, or kidneys, but does increase adrenal weight and accelerate thymic involution. An early transient leukocytosis followed by a severe leukopenia reaching its apogee ten days post injection with a recovery toward normal at fourteen days, has been observed in such estrogen-treated animals.

Aortic deposition of cholesterol in experimental atherosclerosis. A. N. TAYLOR AND JOE A. STEWART (by invitation), Dept. of Physiology, University of Oklahoma School of Medicine, Oklahoma City.

As a part of a study of the flow pattern in major blood vessels, the cholesterol content of four selected regions of the aortae of normal and cholesterinized rabbits was determined. Thirteen rabbits received 0.6 gm. of cholesterol in vegetable oil added to a daily base diet of 75 gm. commercial rabbit chow; 10 rabbits received an equal amount of vegetable oil added to the base diet; and 13 rabbits received only the base diet. Serum levels of total cholesterol, which were determined periodically, averaged at the end of the test period of 100 days, 2000 mg.% for the cholesterinized animals as compared with 60 mg.% for the two control groups. The animals were sacrificed, each

aorta removed and divided into four regions; each region was dried to constant weight, subjected to alkaline digestion, and its cholesterol content determined by Sperry's modification of the Lieberman-Burchard reaction. Cholesterol content of the aortic regions of the vegetable oil-base diet group did not differ significantly from that of the base diet group. Average cholesterol content expressed in milligrams % of the dry weight of each of the four regions was as follows (values for the cholesterinized rabbits are italicized): 1) ascending aorta and arch, *3.47* and 0.17; 2) thoracic aorta below the arch, *1.29* and 0.17; 3) upper abdominal including the renal arteries, *1.50* and 0.23; 4) lower abdominal aorta and proximal iliac arteries, *1.11* and 0.30. A relationship between cholesterol deposition and areas of probable turbulent blood flow is indicated.

Studies of the pulmonary and systemic arterial pressure in cases of patent ductus arteriosus with special reference to effects of surgical ligation. B. E. TAYLOR (by invitation), A. A. POLLACK (by invitation), H. B. BURCHELL, O. T. CLAGETT (by invitation) AND E. H. WOOD. Mayo Foundation, Rochester, Minn.

A study has been made of the blood pressure in the pulmonary artery and systemic circulation in 11 cases of patent ductus arteriosus during surgical ligation of the ductus. Preoperative cardiac catheterization studies were carried out in 4 instances. Pulmonary artery pressures were measured in the open thorax by means of an intra-arterial needle and a strain-gage manometer before and after closure of the ductus. Systemic blood pressures were recorded during the operation by means of an indwelling needle in the radial artery. These pressures and the heart rate, electrocardiogram and respiration all were recorded on the same photographic paper. In 4 patients in whom cardiac catheterization studies were carried out preoperatively, the average flow through the patent ductus arteriosus was calculated to be 7.5 (3 to 17.7) l/min. In 6 patients calculations based on determinations of oxygen saturation of systemic arterial, pulmonary arterial and mixed venous blood indicated that the average flow through the ductus arteriosus was 49 (29 to 75) % of the left ventricular output. The average outside diameter of the ductus arteriosus measured at operation in these cases was 1.1 (0.8 to 1.3) cm., as compared to the average value of 1.0 (0.75 to 1.3) cm. for the complete series. The average mean systemic to pulmonary artery pressure gradient was 60 (16 to 82) mm. of mercury. In these cases there was a direct correlation (correlation coefficient: 0.9 ± 0.25) between the ductus flow in cc/100 cc. of left

ventricular output) and the square of the radius of the ductus multiplied by the square root of the pressure gradient. Closure of the ductus arteriosus produced an immediate increase in the systemic blood pressure and a decrease in the pulmonary arterial pressure in every instance. These changes could be repeated at will by opening or closing the duct with a rubber-covered surgical forceps. In this series of patients the average immediate increase in systemic pressure coincident with closure of the ductus was 8.9 ± 1.9 (3.2 to 20.7) mm. Hg systolic, and 11.9 ± 2.7 (3.1 to 30.5) mm. Hg diastolic. The average immediate decrease in pulmonary artery pressure was 4.3 ± 1.2 (−0.5 to 10) mm. Hg systolic, and 5.8 ± 1.3 (1.1 to 10)mm. Hg, diastolic.

Physiological properties of regenerating nerve fibers. J. D. THOMSON (by invitation) J. A. MORGAN (by invitation) AND H. M. HINES. Dept. of Physiology, State Univ. of Iowa, Iowa City.

Tibial nerves of rats were crushed 1, 2 or 3 times at the same point at 42-day intervals, and gastrocnemius weights and isometric tensions were measured at 21, 42 and 84 days after the final lesion. Values for muscle weight and tension, recorded as percentage of contralateral control, showed that the gastrocnemius can undergo at least 3 consecutive denervations and recoveries without impairment of its regenerative capacity. Twitch tension values in % of tetanus tension (tetanizing frequency 120/sec.) suggest that muscle's ability to summate twitches is not impaired during regeneration, since values at 14–17, 20–22 and 25–28 days are of nearly the same magnitude as control values. The slightly higher values at 20–22 and 25–28 days may mean that optimal tetanizing frequencies for regenerating nerve and muscle are lower than 120/sec., and that if the optimal frequency were used the values might be lower. The rate of transmission fatigue (stimulus frequency 120/sec., ether anesthesia) was much more rapid 21 days after a crush lesion than in normal controls; it was less rapid at 28 days than at 21 days. When intraperitoneal Dial was used as anesthetic, transmission fatigue in normal rats occurred more slowly than under ether; at 21 days of regeneration the rate was more rapid than in controls for the first 5 seconds, then paralleled the control curve from the 5th to 10th seconds.

A comparison of action of anticonvulsants on the excitability of peripheral nerve and of motor cortex. JAMES E. P. TOMAN AND CHARLES D. HENDLEY. Dept. of Physiology, Univ. of Utah School of Medicine, Salt Lake City.

The following observations suggest that anticonvulsants may prevent neuronal alterations produced

by excessive electrical or chemical stimulation without impairment of normal function: *a*) Ringer's solution saturated with Dilantin (diphenylhydantoin) was without notable effect upon threshold, spike amplitude, spike duration, recovery, and conduction velocity of frog sciatic nerve. However, concentrations as low as 0.04 mM/l abolished repetitive responses and extraordinary supernormality produced by brief high voltage shocks. Dilantin also prevented hyperexcitability and repetitive firing in nerves treated with isotonic sodium phosphate solution. These effects occurred with low concentrations of many other anticonvulsants including Phenurone, phenobarbital, Mebaral, Mesantoin, and Epidon. *b*) Dilantin did not affect the exchange of radioactive sodium in frog nerve in Ringer's solution. However, it prevented the increased rate of sodium exchange in phosphate solution. *c*) The frequency-threshold curve for non-convulsive localized movement produced by repetitive brief shocks to the cerebral cortex of the unanesthetized rabbit reaches a minimum at 100 c.p.s. After Dilantin treatment the threshold in the minimum region was not raised significantly, but there were significant increases at the extremes (15% at 1000 c.p.s.; 20% at 3 c.p.s.). *d*) The curve of recovery of responsiveness of the rabbit motor cortex following each stimulus in a repetitive series shows a period of supernormal excitability, with a peak at 2 to 4 msec. Dilantin was without effect on this process. However, Dilantin has been previously shown to prevent high frequency seizure discharges in the rabbit EEG.

Effect of hypophysectomy on the electromyogram. CLARA TORDA AND HAROLD G. WOLFF. *New York Hospital and the Depts. of Medicine (Neurology) and Psychiatry, Cornell Univ. Medical College, New York City.*

Some aspects of myasthenia gravis (e.g. occasional increase of thymus, increase of lymphoid tissue, decrease of acetylcholine synthesis) suggest that the decreased ability of patients with this muscle disorder to maintain an adequate muscle function during prolonged work is a result of a partial dysfunction of the pituitary gland. One of the objective tests characteristic of patients with myasthenia gravis is a decline of the amplitude of muscle action potential during repetitive indirect stimulation. In the following it was ascertained whether removal of the pituitary gland induces a similar dysfunction of the muscle. Therefore, electromyograms of 33 hypophysectomized rats were compared with electromyograms of 20 control rats operated on with the same technic except for the removal of the pituitary gland. Electromyograms were recorded from the gastrocnemius muscle during a few minutes' stimulation of the sciatic nerve with a current having a repetition rate of from 3 to 30 pulses per second and being of 'supramaximal' intensity. The area and amplitude of the muscle action potential of the control rats remained either unaltered or decreased somewhat, e.g. the decrease averaged 19% at the end of a 3-minute stimulation period with 11 pulses per second. The amplitude and area of the muscle action potential of the hypophysectomized rats decreased with low frequency stimulation and the decline of the electromyogram increased with the frequency of stimulation. The decrease averaged 82% at the end of a 3-minute stimulation period with 11 pulses per sec. Therefore, a dysfunction of the pituitary gland may be linked with a muscle dysfunction. This effect is probably not exerted through the adrenal gland alone.

X irradiation of the hypophysectomized rat. E. B. TYREE (by invitation), M. N. SWIFT (by invitation) AND H. M. PATT. *Argonne National Laboratory, Chicago, Ill.*

We reported previously that x-radiation in common with other types of stress appears to result in an increased demand for the adrenal cortical hormone (*Am. J. Physiol.* 150 480, 1947). It seemed of interest, therefore, to determine whether hypophysectomy would prevent the adrenal response to x-radiation and to note whether survival and some of the typical changes in organ weights would be altered under these conditions. Sixty white male rats (200–300 gm). received 750 *r* total-body x-irradiation, 30 having been hypophysectomized one week prior to exposure. Animals were sacrificed at 3 hours and 4 days after irradiation and comparison was made with appropriate groups of nonirradiated rats. Hypophysectomy prevented adrenal changes (decreased adrenal cholesterol concentration and increased adrenal weight) seen in intact rats after x-irradiation, but did not alter the degree nor time course of the splenic and thymic involution. x-ray toxicity appeared to be potentiated by pituitary ablation. Forty-five % of 20 hypophysectomized irradiated rats died 3 to 4 days after the exposure whereas none of the intact irradiated animals succumbed until 6 days after irradiation and there was only a 30% mortality at 16 days. It remains to be determined whether the adrenals of the 7-day hypophysectomized rat do not respond to x-radiation because they are generally less sensitive owing to removal of pituitary influence or because their stimulation after irradiation is mediated solely by the adrenotrophic hormone.

Effect of explosive decompression on the temperature of the lungs. EDWIN G. VAIL (by invitation) AND

FRED. A. HITCHCOCK. Dept. of Physiology, Ohio State Univ., Columbus.

Experiments on explosive decompression conducted in this laboratory, made it desirable to investigate lung temperatures. All experiments were conducted on anesthetized dogs. Lung temperatures were measured with thermistors. A thermistor located in the trachea recorded the temperature of inspired and expired air. The temperature of expired air never reached the temperature recorded in the deep lung. Measurements of temperature at various levels in the pulmonary tract gave a mean temperature in the trachea of 34.5°C, and in the deep lung of 37.1°C. At explosive · decompression, tank temperatures were found to decrease an average of 2.0°C for all terminal pressures. The greatest drop occured in 0.2 seconds. Respiratory patterns showed many variations. The point in a respiratory cycle at which the explosion occured had a pronounced effect on the respiratory pattern. These effects may be due to an exaggerated stimulation of the Hering-Breuer reflex. The average temperature in the deep lung before explosive decompression was 39.0°C, and the average decrease for pressures lower than 220 mm. Hg was 0.5°C. In all experiments following explosion to 30 mm Hg the temperature in the trachea remained at a low level until recompression. The average decrease in tracheal temperature was 8.8°C. Although attempted respiratory movements were observed in these animals no ventilation of the lungs occured. The temperature changes in the lungs occured in two phases. The first phase probably is due to the expanding lung gases, and the second to the vaporization of water.

Duration of anti-secretory action of enterogastrone and urogastrone in rats and a comparison of potency in rats and dogs. F. E. VISSCHER AND M. I. GROSSMAN. The Upjohn Company, Kalamazoo, Mich., and Dept. of Clinical Science, Medical School, Univ. of Illinois, Chicago.

The duration of anti-secretory action of preparations of enterogastrone and urogastrone has been observed in pyloric ligation rats. After a 40-hour fast, adult male rats under ether anesthesia were injected intravenously. Groups of 6 to 8 rats were taken for pyloric ligation under ether anesthesia at different intervals after injection (zero hour, two hour, and four hour). The inhibitory ability of two samples of enterogastrone was greatest during the first two hours after injection; for two samples of urogastrone this action was greatest during the second two-hour period after injection. There was evidence of activity of both urogastrone and enterogastrone during the third two-hour period. The anti-secretory activity of six samples of entero-

gastrone has been compared in the rat and dog. A rat unit is the amount in mg. ·to inhibit by 50% the volume of gastric juice secreted by the pyloric ligation rat; secretion is measured during the first two hours after injection, and compared with uninjected or gelatin injected controls. Values for 50% inhibition are found by interpolation or extrapolation of figures for inhibition (determined in six or more rats at two or more dosage levels). In general, poor correlation was obtained between unitage in the dog and rat, although a dialysed enterogastrone preparation which was most active in the dog was also most active in the rat.

Inferior caval and portal pressures in relation to the formation of ascites in the dog. WADE VOLWILER, J. H. GRINDLAY, AND J. L. BOLLMAN. Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.

Pressures in the inferior vena cava and portal vein of the standing dog were measured with a water manometer attached to flexible polythene tubing previously inserted into these veins at laparotomy. Data were obtained in 5 normal dogs and in 30 animals with previous ligations, constrictions, or anastomoses of the main venous channels around the liver. Ascites, marked hepatic congestion, and engorged hepatic lymphatics were regularly produced by the progressive constriction of the thoracic inferior vena cava from the fibrous reaction to a cellophane band. Most, but not all, of these dogs developed a marked hypoproteinemia with normal albumin-globulin ratio. Inferior caval and portal vein pressure readings in such animals, though elevated, were found to be no higher than in other types of preparations wherein ascites did not occur. Anatomic studies were made of the venous collateral circulation. Attention is called to the anterior spinal veins as important collaterals to the obstructed inferior vena cava in the transfer of blood from the lower cava to the azygos vein. By none of the operations upon the venous circulation was it possible to produce a marked portal hypertension comparable to that which has been reported as measured at the time of laparotomy in some human patients with esophageal varices.

Effects of intravenous histamine on the peripheral circulation in man. K. G. WAKIM, G. A. PETERS, J. C. TERRIER AND BAYARD T. HORTON. Mayo Clinic and Mayo Foundation, Rochester, Minn.

The effects of continuous intravenous administration of histamine diphosphate on skin temperature, blood pressure, heart rate and blood flow were studied on patients who were receiving the drug for therapeutic purposes. The drug was administered to each patient

in a solution of 1:250,000 in saline at successive rates of 0.004, 0.008, 0.016 and 0.024 mg. of histamine/min., respectively. The duration of infusion at each rate was 20 minutes. Control values for skin temperatures, heart rate, blood pressure and blood flow were established before the infusion of histamine was started, and the observations were repeated at regular intervals thereafter for each of the periods of infusion at each of the four infusion rates and for 5 to 15 minutes after the infusion was stopped. The blood flow in all four extremities was determined by means of the venous occlusion plethysmograph with a compensating spirometer recorder. The cutaneous temperatures were recorded galvanometrically by means of skin thermocouples applied to the forehead, to the skin over the right and left deltoid muscles, and over the right and left quadriceps femoris muscles. Histamine produced cutaneous vasodilatation which appeared first over the face and neck of the patient and gradually extended downward over the upper extremities and thorax, reaching the lower extremities only toward the end when the higher rates of infusion were used. There was a definite increase in skin temperature and in heart rate, and a slight decrease in diastolic blood pressure. The blood flow in the four extremities gradually increased in proportion to the dosage used, until at the highest rate of infusion of 0.024 mg. histamine/min. the average increase in blood flow over the control values was 245% in the left arm, 120% in the right arm, 76% in the left leg, and 28% in the right leg, respectively. However, five minutes after the infusion of histamine was stopped, the blood flow averaged only +46% in each of the arms and +40% in the left leg, and +14% in the right leg. The changes in skin temperature, blood flow, heart rate and blood pressure gradually subsided, and the values returned toward the control level shortly after stopping the infusion of histamine.

An analysis of the cardiovascular effects of the intravenous injection of small volumes of hypertonic solutions in the anesthetized dog. WILLIAM W. WALCOTT AND INGRITH J. DEYRUP (by invitation). Dept. of Physiology, College of Physicians and Surgeons, and Dept. of Zoology, Barnard College, Columbia University, New York City.

The marked but transient fall in blood pressure following the intravenous injection of hypertonic solutions in mammals has been ascribed by different investigators to cardiac weakening or, alternatively, to reduction in peripheral resistance (*Am. J. Physiol.* 151: 516, 1947). We have noted that a characteristic diphasic fall in systemic arterial pressure follows the injection of 4 to 20 ml. of 5 to 20% NaCl or 50% glucose

in normal, sympathectomized or vagotomized dogs (nembutal anesthesia), confirming the conclusion of other workers that the hypotension is not reflex in origin. Heart rates varied slightly or were unchanged, whereas the femoral arterial pulse pressure was decreased in the first phase of hypotension, and significantly increased in the second phase. Throughout both phases, pulmonary arterial pressure was slightly elevated or unchanged, pulmonary arterial pulse pressure was increased, and both central and pulmonary venous pressures were somewhat elevated (membrane manometer records). These findings suggest that the first phase of fall in blood pressure may result from myocardial weakening, rather than from decreased venous return or vasodilatation. Vasodilatation must, however, play a part in the secondary fall in mean arterial pressure, when the femoral pulse pressure increases with unchanged or elevated heart rate. It has been noted that a severe, single phase, rather than diphasic, hypotensive response follows the intravenous injection of hypertonic solutions mixed with blood prior to injection. This phenomenon, which is being investigated further, may throw additional light on the mechanism of the characteristic fall in blood pressure after injection of hypertonic solutions.

Effect of low temperature on the mechanical response and action potential of rat muscle. SHEPPARD M. WALKER. Washington Univ. School of Medicine, St. Louis, Mo.

Male rats were anesthetized with 300 mg. of Na barbital/kg. and placed in a cold room until the rectal temperature decreased to about 22°C. The animals were removed from the cold room and the records were obtained from the gastrocnemius muscle by the time the rectal temperature had increased to approximately 26°C. The 'cooled' muscle showed a 50% increase of tension and about a 100% increase in contraction time and relaxation time over the normal muscle in response to single indirect stimuli. The developed tension resulting from 2 shocks with appropriate intervals was always more than double the developed tension of single responses in normal muscle. On the other hand, the developed tension induced by 2 stimuli with similar intervals was usually about 50% greater than the tension developed after single shocks in 'cooled' muscle. Tetanic stimulation at 125 stimuli/sec. for 0.2 sec. induced approximately equal development of tension in 'cooled' and in normal muscle though the rising phase and the falling phase of the tension curve was longer in 'cooled' muscle. The 'cooled' muscles showed only slight (less than 20%) increase of developed tension as a result of treppe in contrast to the 35 to 40%

increase seen in normal muscle similarly exercised. Exercise brought about a 300% increase of relaxation time and a slight decrease of contraction time of 'cooled' muscle. Similar exercise of normal muscle shortened both the contraction time and the relaxation time. Cooling under the condition of these experiments produced about 100% increase of the duration of action potentials obtained with belly and tendon leads.

Effect of the lysozyme on the gastric mucosa. K. J. WANG (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Acute experiments were carried out on fasting rats under nembutal. *Series I:* the rat's stomach was exposed for four to six hours to lysozyme, 2 mg/cc. in buffer *p*H 5.3. Out of six experiments, one rat showed ulcer, one erosion, and a third hemorrhage. In controls the stomach was exposed to buffer alone and no gross or microscopic changes occurred. *Series II:* the mucosa was exposed to lysozyme in buffer and the solution changed every half hour for three hours, followed by 0.2% pepsin in 0.15 N HCl for three hours, changed every half hour. All showed peptic ulcers. *Controls:* one group treated successively with buffer followed by acid-pepsin showed no ulcers. A second control group, treated successively with lysozyme in buffer followed by saline, showed slight erosion or hemorrhage. *Series III:* 0.15 N HCl and 0.2% pepsin and lysozyme together caused some damage in all cases, but definitely less than was caused by the two enzymes in separate solution. *Controls:* the same acid-pepsin without lysozyme, showed only slight change. Besides, the effect of lysozyme on dogs' gastric mucosa has been observed *in vitro* by incubating tissue sections prepared by the freezing-drying method in lysozyme. Most of the mucus in the surface epithelial cells showed vacuolization and the neck mucoid cells were poorly stained. *Controls:* sections incubated in buffer showed intensive stain of both the surface epithelial cells and the neck mucoid cells without vacuolization. It is concluded that the high concentration of lysozyme in buffer at *p*H 5.3 produced erosion and hemorrhage in the gastric mucosa, and increased the injurious action of acid-pepsin.

Sensitivity of the esophagus to the acid-pepsin action. OWEN H. WANGENSTEEN, HENRI SANCHEZ AND Y. SAKO. Dept. of Surgery, Univ. of Minnesota, Minneapolis.

The sensitivity of the esophagus to the action of gastric juice as compared to the sensitivity of the stomach and duodenum was studied. In rats, ligation of the duodenum close to the pylorus leads to ulceration of the esophagus and of the squamous portion of the stomach. These results seem to be enhanced by the added action of histamine. When food protects the stomach of the animal against ulceration, the esophagus might become damaged in some animals. In dogs, ligation of the duodenum close to the pylorus, plus daily injections of histamine, leads to esophagitis and/or perforation of the esophagus when the stomach is still grossly normal. That the distention produced by the ligation is not in itself the cause of the lesions was evident when the ruptured strength of esophagus, stomach and duodenum was tested on normal animals and after ligation. Continous drip of 0.1 Na HCl solution or of gastric juice into the esophagus and stomach lead to ulceration and sometimes perforation of the esophagus while the stomach is still grossly normal.

Motor responses of spatially transposed intestinal loops. D. H. WATKINS (by invitation) AND F. C. MANN. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

Exteriorized skin-covered loops of jejunum and ileum of dogs were studied in respect to their rate of rhythmic contractions and motor responses after feeding. The positions of the intestinal loops were then changed with respect to their distance from the pylorus. Loops which were originally high in the jejunum were transplanted without interference with their blood and nerve supply to a position low in the ileum, and vice versa. Although this operation of intercalary transplantation of intestinal loops carries several inherent risks, several preparations have remained in good condition four years after operation. The rate of rhythmic contractions in the transplanted loops was the same as in the original position minus one or two contractions/min. It is an inherent property of the loop and does not depend upon the relative position of the loop in the intestinal tract. The time of the motor response after feeding depends upon the relative position of the loop in relation to the remainder of the intestinal tract. The more orad the loop, the quicker does the motor response occur after feeding. These findings support the theory that following the ingestion of food, a wave of activity passes down the small intestine.

Function of the pancreas of alloxan diabetic dogs studied by means of cross-circulation experiments. HARRIET R. WEINSTEIN (by invitation), JAY A. SMITH (by invitation) AND PIERO P. FOA. Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago.

In a preceding note cross-circulation studies on insulin secretion were reported. It was found that after an intravenous injection of 20% glucose (5 cc/kg.) into a normal dog (*A*) connected with a recipient dog (*B*) through a pancreatic-femoral anastomosis, the blood sugar of *B* decreases sharply. This was interpreted

as evidence of an increase in insulin secretion by the pancreas of *dog A*. When instead of normal animals, alloxan diabetic dogs are used as donors of pancreatic blood, the blood sugar of *dog B* increases instead of decreasing. If the alloxan diabetic donor and the recipient are connected through a mesenteric-femoral anastomosis the upper glycemia of *B* is much less pronounced and shorter lasting. The results indicate that the hyperglycemia observed in *dog B* is not due to the glucose injected into *A* and carried across the anastomosis, but is probably due to a hyperglycemic substance secreted by the alloxan resistant portion of the pancreas and similar to that found in most insulin preparations. The possibility that the pancreas might secrete a hyperglycemic substance is of great interest as it would explain why depancreatized dogs require less insulin than alloxan diabetic dogs and why a totally depancreatized man requires less insulin than patients with a moderately severe diabetes.

Endocrine influences on renal function. H. L. WHITE, PETER HEINBECKER AND DORIS ROLF (by invitation). Washington Univ. School of Medicine, St. Louis, Mo.

We have previously reported that the depression of renal function following hypophysectomy in the dog and manifested by striking falls in PAH, inulin and urea clearances and in PAH Tm and by a moderate rise in plasma NPN, with normal plasma Na and K levels, cannot be ascribed to thyroid or gonad regression due to loss of thyrotrophic or gonadotrophic hormones. The question of loss of adrenotrophic hormone or of some further anterior lobe principle acting directly on the kidneys or on tissues in general to produce increased renal activity was left unsettled. The present report presents evidence that the renal depression of hypophysectomy is not due to loss of ACTH. First, adrenal replacement therapy with DCA pellets adequate to maintain the observed renal functions of adrenalectomized dogs at or near normal has no protective effect against the depression of these renal functions following hypophysectomy. Second, stimulation of the adrenal cortex of hypophysectomized dogs by ACTH does not improve renal function as it should if the depression were due principally to adrenal deficiency. The inconstantly observed enhancing effects of ACTH on the renal functions of hypophysectomized dogs cannot be ascribed to adrenocortical stimulation, since such enhancing effects are also sometimes seen in adrenalectomized dogs. Furthermore, large doses of whole adrenal cortical extract supplementing DCA pellets do not improve the renal functions of hypophysectomized dogs. It is concluded that the loss of some further anterior lobe principle, not thyrotrophic, gonadotrophic or adrenotrophic, is principally responsible for the renal depression of hypophysectomy.

Use of capacitance changes for the study of the mechanical activity and output of the heart. WILLIAM V. WHITEHORN AND EDWARD PERL (by invitation). Dept. of Physiology, Univ. of Illinois, College of Medicine, Chicago.

The need of a technically simple method of estimating human cardiac output applicable to general clinical and laboratory use is evident. We have begun investigations of the use of capacitance changes in a condenser field containing the heart for this purpose. Using an improved method described in a companion paper, we have now recorded some 100 'cardiodielectrograms' (c.d.g.) on 30 essentially normal subjects. Recordings are technically easy, require a minimum of subject cooperation and yield a beat-to-beat record of cardiac activity. Outstanding difficulty with present apparatus is the necessity of suppressing respiration during recording. Simultaneous recording of phonocardiograms permits interpretation in terms of events of the cardiac cycle. Records reveal a typical general pattern resembling classical cardiometer curves but differing from these particularly in the early and late phases of systole probably because of movements of the heart and the presence of auricles and portions of the great vessels in the field. Consistent individual variations occur. Preliminary calibrations have given values for stroke volumes and cardiac indices compatible with accepted 'normal' values, but conclusions as to validity await further determinations and simultaneous comparisons with other methods. In addition to determinations of output, study of pattern of the c.d.g. in relation to cardiac disease suggests itself as a means of gaining information regarding mechanical activity of the heart comparable to knowledge of electrical activity obtainable from electrocardiograms.

Influence of the extrinsic nerves on intestinal motility. R. M. WHITROCK (by invitation), H. L. TIECHE (by invitation), AND M. H. SEEVERS. Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

The influence of those reflexes which involve the extrinsic nerves controlling the motility of the small intestine of the dog was studied. The local stimulus of balloon distention was used at various intestinal levels. Dogs were prepared in several different ways utilizing innervated and 'denervated' Thiry-Vella fistulae alone and in conjunction with jejunostomies and ileostomies. Spinal anesthesia was used to block functionally the sympathetic system at the cord. Recordings were made by the balloon-manometer method. The following observations were made: 1) distention of the high jejunum, low jejunum and ileum produces inhibition at all small intestinal levels. 2) The inhibition produced is a reflex effect of extrinsic sympathetic nerves. 3) The sympathetic effect is

purely inhibitory. The parasympathetic effect is purely stimulatory, markedly decreased after vagotomy. 4) Sympathetic sensory afferents from the intestine to the cord do not synapse in the celiac ganglion but pass directly to their respective segmental levels in the cord. 5) Vagal afferent fibers do not appear to carry pain sensation; but rather, pain sensation appears to reach higher sensory levels via the sympathetic system and the spinal cord.

Asystolic arterial pressure gradient as a measure of changes of local peripheral resistance. ARNOLD H. WILLIAMS AND HENRY A. SCHROEDER. Dept. of Internal Medicine and Oscar Johnson Institute, Washington Univ. School of Medicine, and Barnes Hospital, St. Louis, Mo.

The total resistance to outflow from the arterial system is composed of a number of resistances in various portions of the body. It is of interest to know the territorial partition of resistance induced by vasoactive drugs and in altered circulatory states such as hypertension. The asystolic arterial pressure gradient is the curve of intra-arterial pressure fall following sudden occlusion of an artery supplying a local circulation. To prove that the gradient is a measure of resistance simultaneous measurements of the gradient and blood flow were made in the femoral or brachial artery of dogs anesthetized with nembutal. Intra-arterial pressure was measured with the Hamilton manometer and flow by a rotameter while collateral blood flow was excluded by wire tourniquets. These gradients did not differ from those obtained under simpler conditions. The combined measurements of pressure and flow furnished an index of resistance with which the changes of the gradient could be correlated. A rough method of estimating resistance changes from the ratio $\dfrac{\text{effective pressure}}{\text{rate of flow}}$ was devised. The asystolic arterial pressure gradient parallels this index of resistance. Therefore the gradient can be used as a measure of resistance. The best method for quantitation that we have found is to measure the slope of the linear portion of the gradient, according to a modification of the Gomez formula for diastolic slope.

Effects of ryanodine on frog muscle. J. H. WILLS AND E. F. MURTHA. Pharmacology Section, Medical Division, Army Chemical Center, Md.

Ryanodine lowers the twitch height and alpha excitability of isolated frog sartorii. Ringer's solutions containing from 0.50 to 3.0 mg.% of alkaloid produced also a temporary decrease in the rest length. The effects on twitch height and rest length precede those on alpha excitability, suggesting that the excitable

system in frog muscle is not the same as the one or ones concerned with mechanical responses. The temporary decrease in rest length appears to involve the expenditure of energy, coinciding roughly with a period of increased oxygen consumption.

Influence of various levels of thiamine intake on maximum work output. MARJORIE WILSON (by invitation), W. W. TUTTLE AND KATE DAUM (by invitation). Depts. of Physiology and Nutrition, State Univ. of Iowa, Iowa City.

A group of 12 women ate a basic diet containing all nutritional requirements except thiamine. The basic diet contained not more than 140 mcg. thiamine/day. During a 6-week control period all subjects ate an adequate weighed diet as established by the Department of Nutrition. During a 6-week experimental period which immediately followed, 6 subjects ate the basic diet (140 mcg. thiamine) and 6 subjects ate the supplemented basic diet (1340 mcg. thiamine). During both periods measurements of maximum work output, a maximum effort one-minute ride on the bicycle ergometer, were taken. A comparison of the means shows no detrimental effects with respect to work output at the end of 45 days for those eating the low thiamine diet. Because the low thiamine diet produced no change, a similar study was designed to extend over a 19-week period to determine if the element of time was important in the manifestation of thiamine deficiency when work output is used as an index. Three levels of daily thiamine intake were established (200, 625, 1000 mcg.) by supplementing a basic diet containing not more than 200 mcg. thiamine. Three subjects were placed at each level. The subjects whose diet contained 200 mcg. thiamine per day suffered a significant decrease in maximum work output at the end of 19 weeks. The data indicate that there were no significant changes in maximum work output for subjects at the 625 or 1000 mcg. levels of thiamine intake.

Normal oxygen saturation of human arterial blood during inhalation of air and oxygen. EARL H. WOOD (with the technical assistance of LUCILLE CRONIN). Section on Physiology, Mayo Foundation, Rochester, Minn.

Roughton and his co-workers (1944) reported that there was a systematic error of approximately 2% in the determination of blood oxygen capacity by the tonometer method, and that this error could be avoided by use of a modification of the method described by Sendroy for determination of blood oxygen capacity directly in the Van Slyke apparatus. Because of the error in the tonometer method, the average value of

95% for normal oxygen saturation of arterial blood, determined by the standard Van Slyke gasometric technic, was 2 to 3% too low, and the resulting value of arterial pO_2 calculated on this basis was approximately 80 mm. Hg, instead of approaching the value of 100 mm. which would be expected if the alveolar to arterial oxygen pressure gradient were very small. These findings have been confirmed in several different laboratories by the use of different methods, and in this laboratory by carrying out simultaneous analyses on a series of blood samples by both the tonometer and Roughton technic. The average normal oxygen saturation of human arterial blood determined in 29 subjects by the technic of Roughton and his coworkers was $97.9 \pm 0.3\%$. Arterial saturation was measured in 16 of these subjects by an *in vivo* equilibration technic described by Comroe and Walker. An average value of $98.6 \pm 0.4\%$ was obtained. Oxygen saturation of arterial blood was determined in 20 subjects during inhalation of oxygen. The average value of $99.1 \pm 0.2\%$ which was obtained indicates that there was a systematic error in the calculation of oxygen saturation of arterial blood under these circumstances. It is believed that this error, as well as the difference in results obtained by the *in vitro* and *in vivo* equilibration technics, arise at least in part from the calculated correction for physically dissolved oxygen, assuming an insignificant alveolar-arterial oxygen tension difference. The data indicate that the average amount of physically dissolved oxygen during inhalation of oxygen was 1.83 ± 0.03 volumes %, as compared to 1.95 volumes % obtained by the conventional method of calculation. This difference is consistent with an alveolar-arterial oxygen tension difference during inhalation of oxygen of 40 ± 9 mm. and could be explained by assuming that approximately 2% of the blood flow by-passed aerated alveoli. Extensive gasometric calibration studies on the Millikan oximeter, a direct reading oximeter, and a whole-blood oximeter indicate that the relationship between the log of the galvanometer deflection and the oxygen saturation is nonlinear. Therefore, the determination of the increase in arterial saturation during inhalation of oxygen indicated by the oximeter on the standard oximeter scale cannot be used as an accurate indication of normal oxygen saturation of arterial blood.

Redistribution of electrolytes (K^{42}, NA^{24}, P^{32}) following electroshock convulsions in rats. DIXON M. WOODBURY. Depts. of Pharmacology and Physiology, Univ. of Utah College of Medicine, Salt Lake City.

The rate of uptake and equilibrium concentrations of sodium, potassium and phosphorus by brain and other tissues were studied in control rats and at various intervals after production of maximal electroshock seizures. The half-times of uptake for brain were: Na 1.2 hours; K two half-times of approximately 2 and 28 hours; and P two half-times of 0.5 and 70 hours. The brain sodium space (or presumptive extracellular space) at equilibrium was determined to be 30% of brain water. The calculated intracellular/extracellular ratios for brain were 28.2 for potassium and 1.77 for phosphorus at 17 hours, but equilibrium was not complete in this period. Immediately following a convulsion there was a reduction of 8.0% in sodium space, which was largely corrected in 20 minutes. At the same time the intracellular/extracellular potassium ratio was reduced by 61%, largely corrected in 20 minutes. The potassium loss from brain cells occurred in spite of a 128% increase in extracellular potassium concentration. A more prolonged increase was found in total brain phosphate, but was associated with a decreased intracellular/extracellular ratio. Although similar changes in sodium space and phosphorus content were found in muscle, liver and spleen, the dramatic loss of potassium was specific for brain. The results suggest an increased peremeability of brain cells to potassium during seizures. Urine and plasma determinations of radioactive sodium give evidence that the hypothalamic-posterior pituitary system is activated to cause retention of water and increased sodium excretion for several hours after a seizure.

Effect of diphenylhydantoin on recovery of various central nervous functions following maximal electroshock seizures in cats. J. WALTER WOODBURY, JESSE SIMONS, ROBERT EVANS, TRUMAN Y. BURTON AND JAMES E. P. TOMAN. Depts. of Pharmacology and Physiology, Univ. of Utah School of Medicine, Salt Lake City.

Duration of post-seizure depression of various central nervous functions mediated at several levels of integration was measured in ten cats subjected to supramaximal electroshock stimulation. The recovery times in chronological order are tabulated below for 31 control tonic-clonic seizures in comparison with 33 purely clonic seizures obtained after treatment with diphenylhydantoin 30 mg/kg. i.p. Diphenylhydantoin treatment reduced the duration of depression of all functions studied. The effect was greater for high levels of integration. There was no significant change in order of recovery as a result of treatment. If duration of depression is an index of intensity of previous convulsive activity, then the data suggest that diphenylhydantoin acts at all levels of the central nervous system to reduce the degree of discharge during maximal seizures. The results do not support the contention that the tonic phase of seizures arises at

subcortical levels or that diphenylhydantoin has a predominantly subcortical action.

FUNCTION	TIME TO RECOVERY, SEC CONTROL	TREATED	TREATED,% OF CONTROL	PROBABILITY
Knee jerk.....	10.7	6.3	59	0.06
Pinna........	13.3	12.9	97	0.2
Corneal.......	13.3	8.5	64	0.001 −
Normal resp...	13.5	8.2	61	0.01
Pupillary light.......	18.3	16.7	91	0.3
Placing.......	35.5	26.7	75	0.001 −
Righting.....	47.2	26.5	56	0.001 −
Pain withdrawal......	53.3	27.8	52	0.001 −
Visual recognition......	61.7	40.7	66	0.001 −
Rage response to pain.....	90.7	68.1	75	0.001 −
Normal locomotion.....	108.0	73.3	68	0.001 −

Heating effects of microwaves with and without ischemia. RALPH E. WORDEN, J. F. HERRICK, KHALIL G. WAKIM AND FRANK H. KRUSEN. Section on Physical Medicine, and Divisions of Experimental Medicine and Clinical Medicine, Mayo Foundation, Rochester, Minn.

Before and after exposure to microwaves, a comparative study was made of the temperatures of the skin, subcutaneous tissue, superficial muscle, and deep muscle of the thigh of the dog with the circulation intact and after artificial ischemia produced by clamping the abdominal aorta. Temperatures produced by periods of 5-, 10-, 15- and 20-minute exposures were measured by means of thermistor and thermocouples. The temperature rises in the ischemic tissues were slightly higher than in the normal tissues but were not considered significant after 5 or 10 minutes of exposure, nor was there any evidence of burning in the experiments performed at these shorter periods. After 15 to 20 minutes of exposure, the increased temperatures in ischemic tissues were considered significant and out of 11 experiments made after exposure for these longer periods, gross evidence of burning was noted in ten. There were several cases in which burning occurred at temperatures that were lower than was seen after exposure of tissues with intact circulation. Temperatures tolerated by normal tissues cannot be regarded as the safe range of tolerance for ischemic tissues. Bony prominences were potential sites for formation of bleb. If an area containing blebs was allowed to cool to control level and again irradiated with microwaves, the temperature of the fluid in the blebs would rise to levels significantly higher than the surrounding

tissues. In addition to the preceding experiments, another aspect of this study was made with trained dogs in order to determine the optimal duration of exposure to microwaves. Periods of 5, 10, 15, 20 and 30 minutes were used. Of these various durations of exposure, the 20-minute period gave maximal heating.

Slow potential changes in the illuminated frog eye. V. J. WULFF (introduced by F. R. STEGGERDA). Univ. of Illinois, Urbana.

It has been often postulated that the electrical changes, which occur in a photoreceptor upon illumination, are instrumental in activating the nervous structures in the optic pathway. Investigation of the validity of this hypothesis has resulted in the following observations: 1) In the grasshopper, the retinal electric response begins a short time after the onset of the light stimulus. The discharge of the optic ganglion begins a short time after the onset of the retinal response. Both of these periods of delay vary inversely with the intensity of the stimulating light, when the exposure is constant. 2) In eye-optic nerve preparations (multi-fiber) of Limulus, the horseshoe crab, similar observations were made. The results differ in one respect namely, that the inverse relation of the retinal-nerve interval (difference between onset of retinal and optic nerve discharge) does not hold for extremely low intensities of illumination. The magnitude of this interval reaches a maximum at moderate intensities and decreases with higher as well as lower intensities of illumination. 3) In the eyes of the frog it was observed that elevated potentials could be recorded during prolonged periods of illumination. The magnitude of these prolonged potentials increased with increasing intensity of illumination. These observations indicate that, in the frog eye, the initial transient changes are followed by a slow and persistent potential, perhaps analogous to Granit's PI.

These observations satisfy the following predictions of the hypothesis stated above: 1) that the latency of the optic ganglion or optic nerve discharge should increase with decreasing magnitude of the retinal electric response; 2) that the potential generated by the retina in response to illumination persists as long as the optic pathway conducts impulses. The latter prediction is apparently fulfilled in the frog eye. In Limulus, however, short but intense flashes of light produces a train of nerve impulse which considerably outlasts the retinal potential.

The physiology of the adenosine triphosphatase of snake venom. E. ALBERT ZELLER. Dept. of Pathology, Univ. of Basel, Basel, Switzerland.

Many local reactions, caused by snake bite, can be

connected with well-definited enzymes of the poisons (E. A. Zeller, *Advances in Enzymology* 8:459–491, 1948), but no clear-cut relationships have been established between the paralytic action of many venoms and their enzymes. Investigation of paralytic principles has revealed a powerful ATP-ase in the venoms of all 16 species of snakes hitherto investigated (as well as in scorpion and wasp venoms). The activity varies from species to species, and reaches the highest values in the case of Bitis venoms ($Q_P = 4000$), which are higher than in any other natural source. The heat-labile ATP-ase liberates one molecule of phosphoric acid from ATP. Glycine, veronal or borate solutions form suitable buffers ($p_H = 8.3$). Magnesium, cobalt, manganese, and calcium activate the reaction, while zinc, cadmium, mercury, copper and iron inhibit it. The enzyme is different from similar ATP-ases of vertebrate tissues. It has been shown by Dr. Leya (Basel) that anaphylactic shock is followed by complete disappearance of the Kurloff-bodies from the blood of guinea pigs. The same happens after the administration of Bitis venom. The addition of zinc (inhibitor) hinders this reaction, as I have shown in experiments performed with Dr. Leya. Thus the ATP-ase of snake venoms seems to be related to the symptoms of shock produced by snake bite. The ATP-ase can be completely inhibited by homologous and heterologous antisnake-venom serums.

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